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POLARIZED LIGHT TECHNIC

A COMPARISON WITH THE MARCHI METHOD

H. E. SETTERFIELD and T. T. BAIRD, *Department of Anatomy, Ohio State University, Columbus, Ohio*

ABSTRACT.—**The authors compare polarized light technic for the study of degeneration of myelinated nerves with the Marchi method. While polarized light shows much the same thing, it has the advantages of being much more rapid, more sensitive, and constant. It does not depend on fixation and staining but on the chemical structure of the myelin substance. These changes in structure begin before the third hour after cutting the nerve of a rat. No further technic is involved beside the making of frozen sections. Photographs are presented to illustrate the method.**

POLARIZED LIGHT TECHNIC

Most histological technics used with nervous tissues are slow, laborious, and often unreliable even in the hands of an experienced person. None of them at their best demonstrate the earliest and minute amounts of degenerative change in the nerve fibers. The method that has been most commonly used in the past has been that of Marchi. It has been assumed by most workers that this method gives fairly constant and reliable results and many studies of degeneration have been based on it. However, recent critical investigations of the method itself by Duncan (1931), Swank and Davenport (1935), and others, have shown that interpretations based on this technic are likely to lead to erroneous conclusions unless carefully controlled by other methods. Prickett (1934) has questioned its value in early or small amounts of degeneration and states that he found difficulty in distinguishing experimental from normal nerves in material where degeneration was supposed to be present.

Swank and Davenport (1935), state that four days after section is the earliest time that degeneration can be demonstrated by the Marchi method. Some of the methods used by Cajal and his students are said to show degeneration within 18 hours after cutting a

nerve. This means that the actual beginning of degeneration has not been demonstrated by any staining method at the present time. Thus it is evident that a more sensitive method is needed if we are to study the earliest stages of myelin degeneration as well as details of its progress. Preferably such a technic should not depend on the artefacts produced by fixation and staining but should make use of such chemical and structural changes as have occured at the time of observation. This may be an impossible condition to expect to find. It is the purpose of this paper to present a method which approximates these conditions and which has already demonstrated degenerative changes within the first three hours after section of the nerve.

The chemical facts on which the Marchi method is based are well known. During degeneration the phospholipids of normal myelin are changed to triglyceride fats. In the latter state they are stained by osmic acid and are, therefore, identifiable as blackened particles in the sheath of the nerve fiber. But in addition to the triglycerides which accumulate during degeneration there appear to be somewhat similar substances produced by fixation, trauma, etherization, and time itself (Swank and Davenport, 1935). These substances stain with osmic acid and are extremely confusing in many cases and undoubtedly have led to erroneous conclusions in the past. These substances have been referred to as "dust" by some writers. Erlholz bodies are apparently another type of structure present in both normal and degenerating nerves. These also stain with osmic acid and still further obscure the actual changes in degeneration.

Polarized light offers a method of observation which we do not believe depends on artefacts and in which the usual artefacts are not visible if present. The pieces of nerves are placed for a minimum of 24 hours in 10% formalin for the purpose of hardening. We have not been able to see that fixing for this length of time in formalin changes the appearance of the fibers in the least. The nerves are then sectioned longitudinally on the freezing microtome at a thickness of 15 to 20 μ. These sections are floated from water onto slides and mounted in a drop of glycerin. Observations are made with a 4 mm. objective in polarized light and between crossed nicol prisms. Since normal myelin is birefringent, it will appear alternately light and dark four times in the rotation of the stage of the microscope. Any isotropic material, which includes degeneration products, is not birefringent and will appear dark at all points in the rotation. Thus any material within the limits of vision at this magnification can be identified as to the presence or absence of

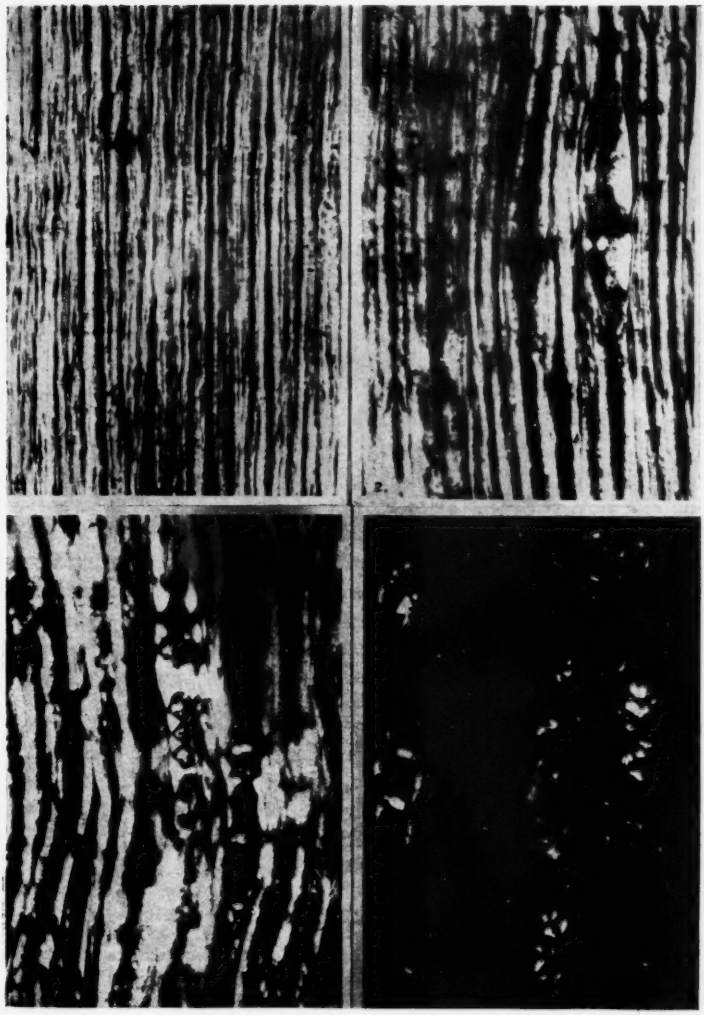

PLATE I

Explanation of Figures

All sections were photographed in polarized light and between crossed nicol prisms.

1. Section of a normal sciatic nerve (450x).
2. Section of a sciatic nerve three hours after cutting (450x).
3. Section of a sciatic nerve twelve hours after cutting (450x).
4. Section of a sciatic nerve twelve days after cutting (450x).

birefringence. Degeneration products begin to appear before the third hour after cutting the nerve as extremely fine granules, so small at first that the field appears cloudy instead of showing discrete particles. These rapidly become confluent during the first six hours so that definite droplets of isotropic fats can be seen in all the fibers. Even before loss of birefringence is apparent there is a very great swelling of the fibers which is plainly visible by this method, but is never seen in dehydrated material.

Degeneration products are not the only isotropic materials in a nerve. The sheath of Schwann, axis cylinder, the nuclei of Schwann cells, connective tissue cells, and certain cytological details of the structure of myelinated fibers are isotropic in normal as well as degenerating fibers.

Since these sections cannot be made permanent, photographs are the only permanent record possible. The photographs reproduced here are selected to show the early changes visible in three hours after sectioning the nerve, as well as more extensive ones occurring during several days. Details of the progress of degeneration in nerves of the rat have been described and published elsewhere (Setterfield and Sutton, 1935). Changes due to various pathological conditions, not degenerative in the usual sense, have been observed and will be published in the future. Also in another paper we will describe in some detail the problem of photography of nerve fibers in polarized light.

Conclusions

The method of polarized light is offered as a supplement to the Marchi and other methods for the study of myelin degeneration. It is simple, rapid, and accurate. It approximates the observation of unchanged nervous tissue and its sensitivity depends more on the skill and experience of the observer than on fixation and staining.

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A NEW METHYLENE BLUE TECHNIC FOR PERMANENT PREPARATIONS[1]

ELBERT C. COLE, *Thompson Biological Laboratory, Williams College, Williamstown, and Marine Biological Laboratory, Woods Hole, Mass.*

ABSTRACT.—**1. Tissues stained *intra vitam* with methylene blue are fixed in a 10% ammonium molybdate solution in physiological saline (or sea water if the tissue is from a marine animal). Fixation time is kept to a minimum. Washing also is reduced to a minimum.**

2. Excess fluids are removed from tissues by blotting with a paper or cloth towel before they are put into the succeeding solution. Tissues are taken from the wash water, blotted and placed in a mixture of equal parts of absolute ethyl alcohol and *n*-butyl alcohol for 30 minutes. They are then blotted and transferred to *n*-butyl alcohol for 30 minutes. After blotting they are placed in a mixture of one part methyl salicylate and four parts xylene until cleared. Tissues may be mounted whole or prepared for sectioning by embedding in paraffin in the usual way.

3. Tissues fixed, washed, dehydrated and cleared as described retain nearly all of the stain; the time required is greatly reduced; there is no need to chill the dehydrating solutions; cell distortion is much reduced.

The value of methylene blue as a vital stain has been considerably restricted by the difficulty of making satisfactory permanent preparations. A loss of stain during dehydration can be greatly reduced by the use of a modified ammonium molybdate solution recently reported (Cole, 1934) which fixes the dye more firmly in the tissues. On the other hand, the use of dehydrating solutions in which methylene blue is less soluble than in the usual ethyl alcohol series seems to be an alternate method. Zirkle (1930) found that *n*-butyl alcohol is of value in eliminating excessive hardening of plant tissues, and that it also facilitates dehydration and clearing of tissues (Zirkle, 1934). The following method, in which *n*-butyl alcohol is used, has given excellent results with vertebrate and invertebrate tissues stained *intra vitam* with methylene blue, when permanent preparations were required.

[1]This paper is the second of a series covering work on methylene blue staining made possible by a grant from the Sigma Xi Society.

Method

Methylene blue[2] was dissolved in physiological saline solution in dilutions varying from 1:3,000 to 1:10,000, according to the tissue to be stained. Frog tissues, such as skin, skeletal muscle or enteric muscle tunic were treated either by injection or immersion. When the desired elements had been stained, the tissue was placed in an ammonium molybdate solution. This was freshly prepared, approximately 10% in concentration, and made up in physiological saline.[3] The best fixation of methylene blue in the tissue was obtained with the minimum immersion in the ammonium molybdate solution, 30 minutes being adequate for small pieces or thin tissues.

The fixed tissue was then washed either in running water or in four changes of tap water for a total period of 30 minutes. Thruout the following steps it was important to remove excess fluid by blotting with a cloth or paper towel before the tissue was transferred to the next solution. After washing, the tissue was put into a mixture of equal parts of absolute ethyl alcohol and *n*-butyl alcohol for 30 minutes or more. It was found that ethyl alcohol alone removed appreciable amounts of the methylene blue, while *n*-butyl alcohol alone prolonged dehydration; the mixture, therefore, of equal parts of these two alcohols at room temperature was tried and proved to give the desired results. Following the bath in the alcohol mixture, the tissue was blotted and transferred to *n*-butyl alcohol alone for 30 minutes. With thick tissues it was usually more satisfactory to dehydrate further in a second bath of *n*-butyl alcohol, again for 30 minutes. It was found to be unnecessary to chill any of the alcohol solutions. After blotting the tissue was transferred directly to a mixture of one part of methyl salicylate and four parts of xylene. As soon as it was cleared, the tissue was mounted whole; or, if sections were desired, it was passed thru two baths of melted paraffin, one hour in each, then embedded in the usual way. Sections were cut from 20 to 40μ in thickness, since the tissue was nearly transparent and the nerve fibers could be studied to advantage in thick sections. A droplet of celloidin-clove oil fixative (equal parts of thick celloidin and of clove oil) was applied to a slide and rubbed to a thin film. Sections were laid lightly on a slide thus prepared, and

[2]Two separate lots of methylene blue were used, both National Aniline products and both certified by the Commission on Standardization of Biological Stains.

[3]The need for careful weighing in the preparation of each fresh solution can be eliminated by the use of "beetleware" measuring spoons procurable at any ten-cent store, and by the computation of the amount of fluid required for approximately a 10% solution.

oriented. They were covered with non-porous paper and gently patted into contact with the fixative. This method permits immediate immersion in xylene. Following removal of the paraffin from the sections, dammar or balsam was added and a coverglass applied.

Study of these preparations reveals that the stain is retained, distortion of cells is slight, and tissues are not excessively hardened. The method is equally satisfactory for frog skin or enteric muscle tunic and for more delicate tissues such as the skin of the squid.[4]

Summary

1. Tissue stained *intra vitam* with methylene blue retains practically all of the dye when *n*-butyl alcohol is used in the dehydrating solutions in accordance with the method described.

2. The time required for dehydration is greatly reduced by the use of equal parts of ethyl and *n*-butyl alcohol.

3. Chilling the dehydrating solutions is unnecessary; these solutions at room temperature extract but little stain.

4. Distortion and rupture of cells is greatly reduced by shortening the time of both fixing and washing, and the tissue is not hardened excessively.

References

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Zirkle, Conway. 1930. The use of *n*-butyl alcohol in dehydrating woody tissue for paraffin embedding. Science, **71**, 103–4.

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[4] In staining and fixing marine animals, sea water or a saline solution isotonic with it was used.

AN IMPROVEMENT IN STAINING TECHNIC FOR PROTOZOA[1]

DAVID L. SARGENT, *Div. of Veterinary Science, Michigan State College, East Lansing, Mich.*

ABSTRACT.—**A modification of Donaldson's iodine-eosin stain for staining intestinal protozoa is presented. This modification consists of using high dilutions of colloidal iodine (Chandler)[2] instead of Lugol's solution as well as high dilutions of eosin. A better resolution of the external and internal structures is brought about by the new method.**

The procedure is as follows: A portion of the fecal material to be examined is suspended in a 0.6% salt solution; the suspension should be of a consistency so that one drop will make a satisfactory microscope mount under a cover glass. To ten parts of this suspension, in a test tube, is added one part of the stain which is prepared as follows:—

10 parts of distilled water
6 parts of a suspension of colloidal iodine (Chandler) containing 4% iodine—20% iodine suspensoid, Merck
1 part of a 10% water solution of anilin red, Merck (eosin yellowish)

Technicians will find, because iodine in the form of colloidal iodine is readily released to the organisms, that the use of this material is far superior to Lugol's solution in carrying out the technic for staining intestinal protozoa in the study of fresh mount preparations. Not only are organisms more deeply stained with iodine but by eosin as well, even when employed in high dilutions.

Donaldson's iodine eosin stain[3] (one part Lugol's solution to one part of a saturated water solution of eosin) has been used for a number of years as a stain for intestinal protozoa in fresh mount preparations. Apparently the function of the Lugol solution is that of killing the organism, thus permitting penetration of the eosin. This, however, gives a uniform red stain with no appreciable degree of

[1]Approved for publication as Journal Article No. 242 (n.s.) from Mich. Agric. Exper. Station.

[2]Chandler, W. L. Iodine as a disinfectant against nematode eggs and larvae. Proc. U. S. Live Stock Sanitary Assoc., December. 1925.

Chandler, W. L. and Miller, E. J. Colloidal iodine. J. Phys. Chem., **31**, 1091–6. 1927.

See also Merck's Index, 1930 edition.

[3]Donaldson, R. An easy and rapid method of detecting protozoal cysts by means of wet-stained preparations. Lancet, **192**, 571. 1917.

differentiation. The endosome and flagellae are seen vaguely, if at all. Apparently the iodine in Lugol's solution is not liberated in sufficient amounts to stain internal structures with iodine, the potassium iodide in the solution being a stronger solvent of the iodine than the organism.

Protozoa are readily killed and stained with suspensions of colloidal iodine (Chandler) and staining of internal structures and flagellae are especially marked, the degree of staining depending upon the strength of the suspension and the length of application. In colloidal iodine suspensions, water is the only solvent present; hence, the iodine in solution readily passes into the organism which has a greater affinity for iodine. Suspended particles of iodine present in excess of that required to produce a saturated water solution quickly go into solution, replacing that which was taken up by the organism.

The transfer of iodine from water solutions to the organism and replacement by dissolution of suspended particles continues until the iodine-fixing power of the organism is satisfied, or until all of the iodine is used up; or, until the organisms are removed from the suspension. Colloidal iodine alone, however, does not bring about a sufficient degree of internal differentiation to permit of satisfactory diagnostic studies in all cases.

If equal parts are used of a saturated water solution of eosin and colloidal iodine, containing an amount of iodine corresponding to that in Lugol's solution (thus simulating the Donaldson stain technic) flocculation of the material in the fresh microscopic mount occurs and prevents accurate observations of the specimen.

If, however, as was recently observed by the writer, higher dilutions of mixtures of both anilin red (eosin yellowish) and colloidal iodine are used, no flocculation occurs and a very marked degree of differentiation is obtained thruout all the structures of the protozoan. The endosome within the nuclei of amoebae, the nuclear membrane with its thickened and thinned portions, as well as the circular and radiating fibrils within the nuclei of certain species of flagellates, are clearly defined.

It seems highly proper that this little discovery be passed on to those who are interested. It is not necessary, however, that a detailed account be given here of the experimentation done with this stain in working out the most effective combinations of stains and dilutions. Suffice it to say that as a result of the above observations, researches were conducted in the Laboratory of Parasitology at Michigan State College and satisfactory combinations and dilutions were worked out.

The writer prepared a series of combinations of the stains which, when applied to the genus *Chilomastix*, revealed a remarkable degree of staining of both internal and external structures. The stains used consisted of a combination of eosin (various forms) and colloidal iodine in high dilutions. The percentage of eosin was unknown, as old materials were used.

Efforts were made to discover the exact combinations of these two stains necessary to bring about the most satisfactory results. A fresh sample of "eosin yellowish" (water soluble) was obtained and a large number of mixtures of various percentages of each component were prepared and applied to both fresh and preserved specimens of several genera of parasitic protozoa, including amoebae, flagellates, and coccidia. Thru careful observations and a process of elimination, correct percentages of each stain were discovered and a stain developed which gave results even more effective than the original combination.

After the formula was determined, it became the purpose of the writer to resolve the facts into simple but accurate directions for the use of this stain which can be readily followed in any biological or hospital laboratory. The first step is to collect and preserve the fecal sample. The tecnic as developed by Dr. W. L. Chandler is as follows:

The patient should be instructed, or the physician advised to instruct the patient, to drink a one-half pint bottle of citrate of magnesia just before retiring. The next morning about eight o'clock or so, regardless of the number of evacuations during the night, the patient should be instructed to collect a stool sample directly into a pint or one-half pint fruit jar and without urine contamination. This sample should reach the laboratory within one-half hour after being voided.

The laboratory technician should heat to body temperature about 50 cc. of a 0.6% salt solution in a small Erlenmeyer flask, and a sufficient amount of the stool sample should be immediately thoroly fragmented in this warm salt solution, using a sufficient amount so that a drop will make a microscope mount that readily can be studied.

If the stool sample is solid or semi-solid, the transfer to the salt solution can be made by means of a mushroomed glass rod, making as many transfers as is necessary to bring about the desired suspension. If the stool sample is liquid, it can be transferred to the salt solution by means of a medicine-dropper, or pipet provided with a medicine-dropper bulb.

The salt solution suspension of the fecal material should then be iodized by introducing as rapidly as possible, drop by drop, colloidal iodine containing 4% iodine, using enough so that a faint iodine color is still present at the end of two minutes. The saline suspension should then be filtered thru cheesecloth. This can best be accom-

plished by using a small beaker into which to filter the material. The cheesecloth should be wet with water in order to facilitate filtration. To this filtered material should be added an amount of formalin sufficient to make a 5 to 10% formalin solution. The material can then be transferred to a bottle for shipping or stored for further observation. Materials collected and preserved in this manner have remained in good condition at this laboratory over a period of years.

The object of the warm saline solution is to encourage motility of the organisms, then if the organism is immediately killed, which can be done by the use of colloidal iodine, the motile characters are preserved. As an example, if motile *Chilomastix* species are killed slowly they have a tendency to round off and the principal characteristics are lost; also if amoebae with extended pseudopodia are killed slowly they retract these pseudopodia. If the iodization is properly done the organisms are killed almost instantly and the typical characters of motile forms may be observed in the preserved material.[4]

To ten parts of such a preparation add one part of the following stain mixture:

10 parts of distilled water
6 parts of a suspension of colloidal iodine (Chandler) containing 4% iodine.
1 part of 10% water solution of eosin yellowish[5]

The ten parts (drops or cc.) of water should be placed in a glass stoppered bottle, the colloidal iodine added and the mixture agitated; and then the eosin Y solution added and the mixture again agitated.

This stain combination apparently does not deteriorate with age, but should be thoroly shaken before use. It can be used for immediate study of fresh preparations or stained preserved material can be kept indefinitely. The degree of differentiation of the organism's structure is increased for a period of ten days after adding the stain and then remains in a satisfactory condition permanently.

[4]Laboratory instruction sheets, Bacteriology 307, Michigan State College. 1935.
[5]Merck's eosin yellowish employed by writer.

THE PHYSICAL CHEMISTRY OF SILVER STAINING

LEO ZON, *Department of Pathology, University of Minnesota, Minneapolis, Minn.*

ABSTRACT.—**It has been shown that silver deposition plays a part in the silver staining process. From this it has been concluded that the rate of reduction of silver within and on histological structures is an important factor.**

Some factors controlling the rate of reduction, such as the adsorption of silver hydroxide and ammonia, the affinity of silver for proteins, and the protective power of the gel structures have been pointed out.

Some simple applications of the ideas to silver staining have been given and two technics described, one making use of piperidine instead of ammonia, the other carrying out the reduction in the presence of the silver solution to facilitate deposition.

HISTORICAL

Cajal (1903) believed that tissues contained albumens which united with silver and that the resulting silver compounds could be easily reduced, thus making structures visible. In 1920 he made the suggestion that in silver ammino-carbonate solutions an "ammoniacal oxide" of silver is produced which is taken up by the histological elements in a selective manner. He gave no data, however, on this point.

Bolsi (1927) presented a view that was essentially an elaboration of Cajal's idea. He believed that the silver salts are selectively adsorbed by tissue elements.

Liesegang (1911) suggested that Cajal's stain is analogous to the photographic process. He drew the analogy in the following manner: While the tissue is in the silver nitrate solution, by virtue of some reducing action of the histological elements, some silver is reduced, and this forms the nuclei as in the exposed photographic plate; upon subsequent treatment with hydroquinone these nuclei have silver deposited upon them and outline histological elements.

Cajal (1921) discussing Liesegang's theory, accepted the principle of the "deposition" character of the stain but offered two objections to the nucleus idea. The first was that treatment of tissues with oxidizing agents did not change the staining; the second, that the theory did not explain what he called the "transference of nucleation". Thus, as he pointed out, when tissues were treated with various reagents, different structures were stained.

Kubie (1929) suggested that when tissues are placed in the reducing bath following immersion in silver ammino solutions, silver salt tends to diffuse out and, depending upon the speed of reduction, silver will be deposited either in the tissue or upon its surface.

Visintini (1931) thought that the reduction reaction forms a silver sol and that this sol is then specifically flocculated upon histological elements.

The Deposition Nature of the Staining Process

It is the aim of this paper, first, to show that an essential feature of the staining process is the deposition of silver upon the stained elements and, second, to discuss some of the physical and chemical factors influencing this deposition. Before proceeding with this, it is necessary to discuss briefly the chemical compounds used.

The Chemistry of the Silver Ammino Compounds. Reychler (1895) showed that when ammonium hydroxide is added to silver nitrate, first silver hydroxide is precipitated and then, as more base is added, the silver hydroxide redissolves to form a compound $Ag(NH_3)_2NO_3$. The exact amount of ammonia required to make a clear solution is given by various authors: Herz (1910) gives 2.12 moles of ammonia for each one of silver; Kubie and Davidson (1928), 2.06 at 25°; Euler (1903), 2.08.

The state of equilibrium in a solution of silver diammino-nitrate is given by Kohlschutter and Fischman (1912) as follows: The salt ionizes completely into a complex cation $Ag(NH_3)_2$ and the nitrate anion; the complex cation dissociates slightly giving silver ions and by hydrolysis ammonium and hydroxyl ions; the excess of ammonium hydroxide above 2 moles for each one of silver is necessary to depress the number of silver ions so that silver hydroxide will not be precipitated. A complete diagram of this equilibrium is given by Kubie and Davidson (1928).

Euler has pointed out that the compounds formed by silver nitrate with methylamine and ethylamine are analogous in every way with that formed with ammonium hydroxide. It is assumed that piperidine acts in the same way as these amines. These amines are stronger bases than ammonia, and the equilibrium conditions of their complex silver salts are different from that of silver diammino-nitrate. Table 1 gives the dissociation constants of the complex cations, calculated from the pH, as done by Euler.

These figures indicate that the compounds formed with ethylamine

TABLE 1. DISSOCIATION CONSTANTS

Base used to form ammino-compound with $AgNO_3$	Molarity of silver solution	Ratio of base to silver in moles	pH measured with indicators	K calc. from pH	K calc. from silver electrode
Ammonia	.05	2.09	9.9	1.3×10^{-8}	1.5×10^{-8} (Euler)
Ethylamine	.05	2.20	10.9	1.0×10^{-7}	2.5×10^{-7} (Euler)
Piperidine	.01	3.60	11.8	5.4×10^{-6}	No data

and piperidine will behave differently from the ammonia compound in regard to their reduction, adsorption, diffusion, and possibly, therefore, also in their staining properties. Other amines which form complex silver compounds such as diethylamine, *n*-butylamine, ethylenediamine were tried in stains with less success. The long chain amines separate into an oily phase and cannot be used.

Experimental Proof of Deposition. If it can be definitely shown that a large fraction of the metallic silver present on or in a stained element is deposited during the reduction, then the simple view advocated by Cajal and Bolsi must be altered.

To each solution in Table 2 was added 0.5 g. of raw Japan silk fibers. After 2 hours aliquot samples of the silver solutions were withdrawn and titrated in nitric acid with ammonium thiocyanate, using ferric ammonium sulfate as an indicator. From this titration the amount of silver taken up by the fibers was calculated. To each flask 0.5 cc. of 37% formaldehyde solution was added. The time for reduction to start, as indicated by the presence of reduced silver, was noted. After two hours the fibers were removed and washed with large quantities of distilled water until all the coarse particles of silver mechanically held in the meshes of the fibers were removed. The fibers were then washed in sodium thiosulfate to remove any unreduced silver salts and again in distilled water. The metallic silver staining the fibers was dissolved in concentrated nitric acid and titrated. Table 2 shows that in all but two cases the amount of silver finally present was greater than that held adsorbed before the reduction. We must conclude then that during the reduction process metallic silver is deposited upon and in the protein fibers.

If the fibers are removed from the silver solution and added to a formalin solution, the results are different. The staining becomes a race between the process of reduction and the process of diffusion. Using 10% formalin it was noticed that the fibers from all the solutions stained well, except the fibers in solution No. 4. Here, as can

be seen from Table 2, the reduction was slow so that silver diffused away from the fibers before reduction could occur. Similar experiments, using frozen sections of brain tissue in place of fibers, showed that 1.3 to 2 times the adsorbed silver was finally present in the tissue. Here the frozen sections were removed from the silver bath and reduced one by one in 5% formalin.

A simple histological observation upholds the deposition idea. Clear areas about deeply stained nuclei as in Fig. 3 (Plate I) are often seen. It may be noted that axis cylinders which come close to these nuclei are unstained, indicating mobilization of silver.

TABLE 2. DATA INDICATING DEPOSITION OF SILVER ON FIBERS

Content of solution 50 cc. volume	Adsorbed silver (grams)	Silver found on fibers	Time for reduction to begin	Character of solutions after reduction	Color of fibers after reduction
1) 4.9 cc. .1N $AgNO_3$ 4.9 cc. 2.1N NH_4OH Contains .052 g. Ag.	.010	.028	10 min.	Clear with brown tint	Black
2) 4.9 cc. .1N $AgNO_3$ 4.9 cc. 2.3N ethylamine Contains .052 g. Ag.	.015	.033	5 min.	Slightly brown, no precipitate	Black
3) 4.9 cc. .1N $AgNO_3$ 4.9 cc. 3.6N piperidine Contains .052 g. Ag.	.022	.022	Immediate	Black with precipitate	Brown
4) 4.9 cc. .1N $AgNO_3$ 9.8 cc. 2.1N NH_4OH Contains .052 g. Ag.	.010	.032	30 min.	Clear	Black
5) 4.9 cc. .1N $AgNO_3$ 4.9 cc. .05N Na_2CO_3 4.9 cc. 2.1N NH_4OH Contains .052 g. Ag.	.011	.030	3 min.	Brown	Gray
6) 4.9 cc. .1N $AgNO_3$ 4.9 cc. .1 N NaOH 9.8 cc. 2.1N NH_4OH	.021	.017	Immediate	Black with precipitate	Brown-yellow

The mechanism of deposition in the formation of mirrors has been studied by Kohlschutter and Fischman. They showed that reduction of silver goes on rapidly at surfaces because of the strong adsorption of both formaldehyde and silver hydroxide. As silver is deposited more silver hydroxide is adsorbed and reduced. We see, therefore, that the faster the reduction goes on at the surface in relation to the reduction in the body of the solution, and the more time given for diffusion to take place to the surface, the greater the deposition will be. The results shown in Table 2 agree with

this conception. In the case of the fibers, the slower the reduction the more deposition. In solutions 3 and 6, Table 2, the solutions surrounding the fibers were so rapidly reduced due to the high pH that no time was allowed for diffusion to the fibers. In tissue staining, as in the Bielschowsky technic, there will be a race between reduction and diffusion, so that if the reduction is too slow, no staining takes place.

Summing up, we can say that deposition must play a part in staining and that the deposition depends upon the rate at which the reduction reaction occurs on and within histological structures.

Factors which Influence the Rate of Reduction

Since the deposition depends upon the rate of reduction at the surfaces of the histological elements, we are interested chiefly in those factors which are operative at these surfaces rather than in general factors, such as temperature. A brief glance at the general nature of the reduction reaction is first necessary.

When formaldehyde is added to silver diammino solutions we should expect a quantitative formation of hexamethylenamine, since free ammonia is present. This should lead to the formation of silver hydroxide which could react with formaldehyde still present. At the same time formic acid is produced. The conditions are so complex that we cannot in any way predict which of the above reactions will proceed more rapidly. In general, there is an excess of formaldehyde present in silver staining so that the removal of formaldehyde from a solution in the form of hexamethylenamine need not worry us.

Measurements by the writer indicate that the time course of the above reaction is given by a sigmoid curve, indicating a catalysis. Some experiments by Kohlschutter and Fischman indicate that the metallic silver formed may be the catalyst.

The presence of reducing substances in tissues which might form catalytic "nuclei" of silver has been demonstrated by Loew and Bokorny (1881) Masson (1928), Kon (1933), and others. It might be thought that it would be easy to manipulate these nuclei by means of the well-known procedures of photography and thus determine their influence. As has been pointed out by Cajal, the results of such a manipulation are inconclusive. We can only add that if such nuclei are formed in tissues, they must be highly protected by the gel structures and are, therefore, of secondary importance. The autocatalytic nature of the reduction will tend, however, to accentuate any differences in the initial rates of reduction.

The adsorption of silver and formaldehyde. From Table 2 it may be noted that silver is removed from solutions by protein fibers. It is important to know in just what form silver is removed.

Silver ammino solutions were allowed to stand two hours with samples of wool yarn. The silver adsorbed was determined by titration of an aliquot sample. The carbonate was measured in the Van Slyke apparatus. The total base adsorbed was determined by titrating an aliquot sample with nitric acid, using methyl orange as an indicator. The ammonia was determined by adding strong sodium hydroxide to the aliquot sample and distilling it into a measured amount of standardized acid.

Experiment I			Temp. 24°C.
Solution: Silver diammino-carbonate		Ag. conc. molarity	CO_3 conc. molarity
Before adsorption	No. 1	.095 M	.05 M
	No. 2	.090 M	.044 M
After adsorption	No. 1	.026 M	.049 M
	No. 2	.050 M	.042 M

Similar results were obtained when silver ethylamine carbonate was used.

Experiment II				Temp. 23°C.
	Solution: Silver diammino-nitrate			
Adsorbent	Initial conc. Ag. sols.	Millimols Ag. adsorbed	Millimols NH_3 adsorbed	Millimols Base adsorbed
0.5 g. wool	.02 M	.51	.18	.71
0.5 g. wool	.02 M	.51	.175	.70
0.5 g. dry brain tissue	.02 M	.24	.16	.37

Experiment III				Temp. 23° C.
Adsorbent	Amine used to make complex with silver nitrate	Millimols Ag. adsorbed	Millimols base adsorbed	pH
0.5 g. wool	Ethylamine	0.58	1.48	10.8
0.5 g. wool	Piperidine	0.74	1.68	11.7

From these figures one may conclude: (1) that the anion is not taken up (Exp. I), (2) that ammonium hydroxide and silver hydroxide are adsorbed since the base equals the silver plus the ammonia (Exp. II), (3) that the ratio of silver to base varies as different compounds are used (Exp. III). Since base may buffer the formic acid produced by oxidation of formaldehyde, and since ammonia may react with adsorbed formaldehyde to form hexamethylenamine, the

ratio of base, amine, and silver must play an important role in controlling the rate of reduction of silver. The conditions are so complex that it is impossible except by experiment to tell what the optimum ratios for deposition are.

The writer has studied the adsorption of formaldehyde upon silk and wool fibers. As no definite equilibrium is reached in several days, no exact figures can be given. The adsorption is great, however, for in low concentrations large fractions of the total formaldehyde disappear from the solution. This is consistent with the finding that surface active agents, such as ethyl alcohol, saponin, and butyl alcohol, do not greatly inhibit the deposition of silver in such experiments as were presented in Table 2. These agents are thus unable to displace formaldehyde from the protein fibers.

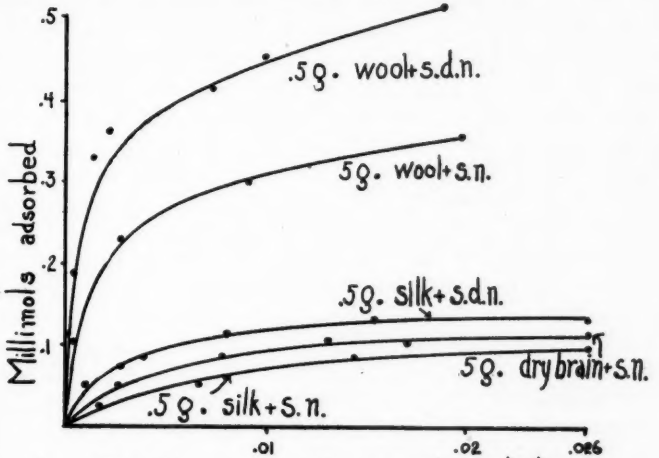


Fig. 1.

Since the majority of commercial samples of formaldehyde contain large percentages of methyl alcohol and also some formic acid, fresh solutions of formaldehyde were made by dry distillation of paraformaldehyde. These solutions were standardized according to the method of Romijn (1897). In the staining of tissue only these solutions were used.

The influence of concentration and the affinity of silver compounds for fibers. It might be at once expected that the amount of silver compound taken up by the structures would influence the rate of reduction. The amount taken up depends upon the concentration

in the surrounding solution, as may be seen from Fig. 1. The results, when plotted upon chained coördinates, gave straight lines. This indicates that the union follows the Freundlich adsorption isotherm.

We may reason as follows: Adsorption of silver occurs only because a decrease in free energy takes place. As the concentration increases, the adsorbent becomes saturated and successive increments of adsorbate cause less free energy change and are held less avidly. The rate of reduction, therefore, of adsorbed silver compounds should increase rapidly as the concentration of the silver solution increases.

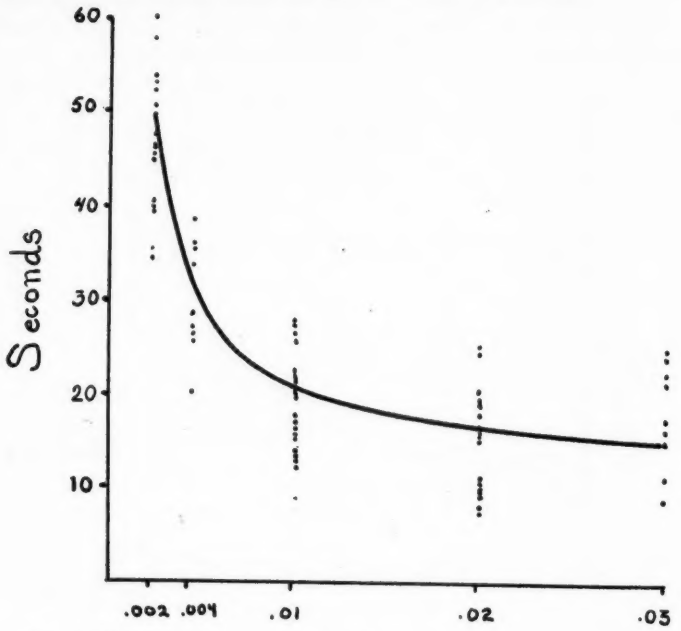


Fig. 2.

This idea was tested in the following manner: Silk fibers were immersed in varying concentrations of silver diammino-nitrate. The fibers were removed from the silver bath and placed upon a microscope slide, the excess solution being removed with a piece of filter paper. A full strength solution of formalin (37%) was then dropped upon the fibers. The fibers were watched with the low power of the microscope and the time for the occurrence of the first definite brown

color was measured with the stop watch. The results of such an experiment are given in Fig. 2. The curve plotted by inspection shows the trend. It is in agreement with the above idea.

Control experiments show that between molarity of 0.002 to 0.05, reduction occurs, i.e., a brown tint appears in the solutions in from 25 to 35 seconds and is independent of dilution. This is what might be expected when the reacting agent in these ammoniacal solutions is silver hydroxide. When dilution occurs the ammino-compound undergoes greater hydrolysis, and the amount of silver hydroxide remains almost the same.

From Fig. 1 it may be seen that at lower concentrations wool fibers are relatively less saturated than silk fibers. We expect then that at these concentrations the staining of wool would be more difficult than that of silk. At saturation concentrations we should expect that the staining would become about the same for the two types of fibers, for the affinity of the last increments of silver would approach zero in both cases. Experiment shows, however, that in concentrations of 0.03 M, i.e., close to saturation (Fig. 1), silk fibers are stained while wool fibers remain entirely white, altho they contain about four times the silver of the silk fibers. The staining was done by transferring the fibers from the silver solutions without washing into 1% formalin. Upon this basis we may conclude that the difference in staining properties between silk and wool depends upon some factor other than their affinities for silver compounds.

The role of protective action. Reinders and Van Nieuvendurg (1912) showed that protective colloids inhibit the reduction of silver nitrate by ferrous citrate. Williams and MacKenzie (1920) found that gelatin retards the precipitation of silver dichromate for many hours. In both cases the retardation is ascribed to the slowing of the rate of crystallization. Because of this influence the precipitation of an insoluble substance occurs more rapidly in one gel than in another.

If we add to 5 cc. of a 0.5% solution of iso-electric gelatin, 0.5 cc. of 0.05 normal silver nitrate and then add 0.3 cc. of 0.05 molar potassium bichromate, silver dichromate will be precipitated in several hours. If silk and wool fibers are added to this solution, some fibers take on a distinctly red color long before the surrounding solution becomes tinted red by silver dichromate. Microscopic examination shows that the silk fibers are stained deep red by a very fine and even deposit of silver dichromate. The wool fibers are colored a light yellow and have apparently taken up potassium bichromate. The interpretation is as follows: The gelatin cannot diffuse into the fibers. The salts can do so easily. The protective power of silk fibers

being less than that of the surrounding gelatin solution, the precipitate starts within these fibers sooner than in the surrounding solution. This precipitate once formed grows by diffusion. The protective power of the wool fibers being greater than that of the surrounding solution, no precipitate is formed. Differences in density do not explain the difference in staining, for in this case there should at least be a precipitate upon the surfaces of the wool fibers. In carrying out these experiments with other samples of gelatin it may be necessary to vary the concentrations given. The important point is that the precipitate should form slowly over a period of hours.

A frozen section of formalin fixed brain tissue was placed in a 2% silver nitrate solution for 24 hours. It was then washed with distilled water and placed in a test tube containing 3 cc. of 1% gelatin solution. To this solution was added 0.04 cc. of 0.1 molar silver nitrate and 0.2 cc. of 0.1 molar potassium dichromate. The test tube was left over night in an incubator at 37°C. Fig. 4, Plate I, shows a section stained by this method. Nerve fibers are clearly and sharply shown, altho they are not completely stained. There is no diffuse coloring of the tissue.

Upon the above basis and from the results of the silver staining experiments, we can assume that nerve fibers in company with silk fibers stain easily with silver because their gel structure allows precipitation of metallic silver to occur readily. A simple observation supports this view. It can be noted in silver stained sections that the colors assumed by tissue elements are exactly those that may be seen in silver sols of different particle size. Nageotti and Gyon (1930) have shown by observation with the cardioid condenser that silver stained structures contain colloidal particles of silver. Von Weimarn (See Gortner, 1929, p. 14.) has shown that the degree of dispersion of a sol is dependent upon the ratio of the rate of nuclear formation to the rate of crystal growth. Since the gel structure can affect the rate of crystal growth the above color variations are exactly what should be expected, if each structure has a different protective power.

The conception of the Golgi stain based upon the above considerations is as follows: The brain substances form a non-homogeneous gel. When the tissue is immersed in potassium dichromate it takes up a certain amount of the chromium salt. The silver nitrate diffusing in produces a supersaturated solution of silver dichromate. Precipitation begins in the regions where protective power is least. More silver dichromate diffuses to those regions and is added to the precipitate already present.

Many facts uphold this theory. When liver is stained by the Golgi method, the bile canaliculi often stand out sharply. This is exactly what we would expect since the canaliculi presumably being spaces, would fill up with precipitated silver dichromate, while the cells being protective gels would have only a finely dispersed precipitate. Frequently the capillaries of the brain are shown beautifully outlined. Careful examination shows that the structures seen are solid rods of silver dichromate precipitate. The lumen of the capillaries is filled up in the same manner as the bile capillaries.

Practical Applications

If it is true that silver staining is at least in part a process of deposition, then the most favorable conditions for staining should occur where no diffusion away from the tissue is possible and where diffusion to the tissue is possible. This condition occurs if we carry out the reduction process in the presence of the silver compound.

The section shown in Fig. 5 (Plate I) was stained by the following procedure: The frozen section of formalin fixed tissue was placed in a test tube containing 1 cc. of 0.05 molar sodium carbonate, 1 cc. of 0.1 molar silver nitrate, and 1 cc. of 0.209 molar ammonium hydroxide. To this was added 0.05 cc. of 0.08 molar formaldehyde solution freshly made from paraformaldehyde. The tissue gradually became dark while the surrounding solution remained clear.

In the procedure just described staining occurs long before any colloidal silver is produced in the solution surrounding the frozen section. The silver sol must, therefore, be produced in such intimate relation to the tissue that no silver particles diffuse away. The staining cannot then be analogous to immersing tissue in sols, and the particle size does not determine what structures are stained, as held by Visintini (1931) but, on the contrary, the structures determine by their properties the size of the colloidal particle produced within them.

The above process is so simple that if standard solutions are made up we can repeat the staining in identical fashion time after time. When frozen sections from a large series of brains, all supposedly fixed in an identical manner, are stained, wide variations are seen. Fig. 6 is an example of a result often obtained. The nuclei stand out black upon a bright yellow background showing no detail. This section was stained together with one from the material shown in Fig. 5 as a control. Fig. 7 shows a section taken from the same block as the section in Fig. 6, stained after 4 days' additional fixation in fresh 2% formaldehyde. Fixation in 10% silver nitrate for several days often causes the same change. Fixatives not only

PLATE I

Fig. 3 is a frozen section of formalin fixed white matter from the cerebrum. The section was stained by the Bielschowsky technic, using silver ammino-nitrate and sodium hydroxide with a 5% formalin solution as the reducing agent. The clear areas about the nuclei are thought to be due to a mobilization of silver from the surrounding areas. ×200.

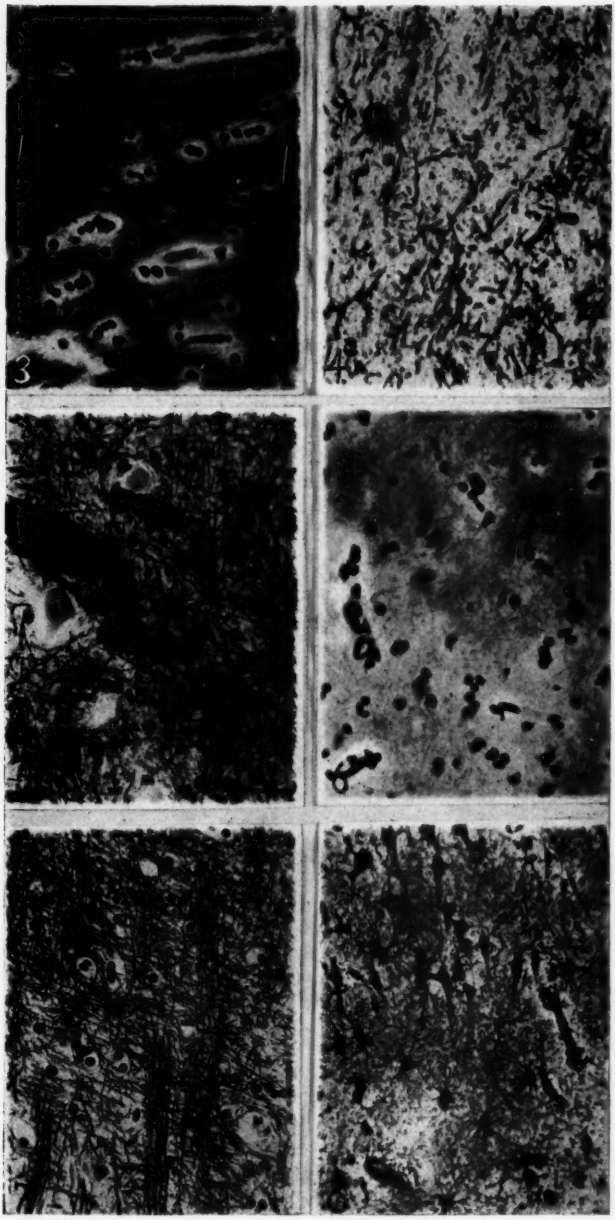
Fig. 4 is a frozen section of formalin fixed white matter stained with silver dichromate as described. This section shows that the silver dichromate is deposited upon the nerve fibers but nowhere else. ×200.

Fig. 5 is a frozen section of formalin fixed white matter from the cerebrum. The section shows the sharp staining of fibers obtained by the modified procedure of allowing the reduction to take place in the silver solution. ×200.

Fig. 6 is a frozen section of formalin fixed white matter from the cerebrum. The same staining procedure as in Fig. 5 was used. The nuclei stain deeply while the nerve fibers are light yellow with no detail. The yellow areas appear as white in the photograph. This section represents a not uncommon result obtained with silver stains. ×200.

Fig. 7 is a frozen section from the same material as shown in Fig. 6. The frozen sections were allowed to remain in a 2% formalin solution for four days before staining. The staining procedure was the same as in Fig. 5. The section shows a marked contrast to Fig. 6 in that the nerve fibers are sharply and accurately stained. ×200.

Fig. 8 is a frozen section of gray matter from the same material as Fig. 7, stained with piperidine silver carbonate as described. This is a fairly typical result obtained with this stain and shows the peripheral glia and several microglial cells as well as the pyramidal cells of the cortex. ×300.



change the protective power of gels but also their ability to adsorb silver. It can only be indicated here that the fixation and condition of the tissue is probably the most important variable in silver staining.

It was indicated before that when the silver solutions are made up with ethylamine and piperidine, the ratio of silver and base adsorbed is different from that when ammonium hydroxide is the base. We must assume that different histological structures are affected differently by this change in adsorption so that there may be a differential effect upon staining as well as a general one. Fig. 10 is a frozen section stained by the following procedure: To 1 cc. of N/10 silver nitrate was added 1 cc. of 0.05 molar sodium carbonate and the precipitate was dissolved by the addition of 0.2 cc. of piperidine. The frozen sections were washed first in distilled water, allowed to remain in the silver solution from 1 to 3 minutes, and transferred without washing to 50 cc. of 0.05 molar formaldehyde solution.

Upon the basis of all the foregoing considerations we can offer the following conception of the Bielschowsky type of stain: The tissues take up ammonium hydroxide and silver hydroxide in various ratios. Each histological structure has a different affinity for the silver and also a different protective power. In the reducing solution adsorption of formaldehyde takes place and, depending upon the above factors, reduces silver hydroxide either slowly or rapidly. Those structures in which reduction of silver hydroxide proceeds fastest will acquire a deposit of silver from the solution carried over mechanically from the silver bath. This mechanically held silver will tend to diffuse away and if the reduction is too slow, will be unavailable for deposition.

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NOTES ON TECHNIC

STAINING WITH SAFRANIN AND FAST GREEN FCF.—Until within the last few years, the visual advantages of a red-green differential stain to bring out vascular tissue in contrast to non-vascular tissue have been offset by the fact that no permanent green has been available. The introduction of fast green FCF, which is an apparently permanent stain,[1] has made possible staining schedules giving red for xylem, and green differentiation of the other tissues, with a minimum of difficulty and a maximum of differentiation.

The following schedule is recommended by the writer for general use. It produces excellent slides, which have good visual contrast as well as excellent differentiation. We have used it to stain series of sections for floral anatomy, miscellaneous stems, leaves, and roots, which had been preserved in a variety of fluids. Some of the graduate students have tried it on celloidin material, and on wood sections, and have secured good results.

The following stock solutions are made up beforehand:[2]

A. Safranin, 1% in 50% alcohol. (This is used diluted, 20 drops per Coplin jar, or about 2 cc. stock to 40 cc. tap water. Use once.)

B. Fast green FCF, 0.5% in absolute alcohol.

STAINING SCHEDULE

1. Remove paraffin and hydrate to 70% alcohol. Xylol, 5 minutes; rinse in equal parts xylol and absolute alcohol; absolute alcohol, 5 minutes; 95% alcohol, 5 minutes; 70% alcohol, 5 minutes.

2. Put 20 drops of stock safranin into each Coplin jar. Add sufficient tap water so that the slides will be covered. Discard this solution after it has been used.

3. Place slides in safranin and stain. The time may be varied and naturally will vary with the material. Thirty minutes should be considered a minimum; 1 or 2 hours is enough in many cases; while if it is more convenient the slides may remain in the stain during the day or overnight without overstaining.

4. From this point on handle the slides 1 or 2 at a time. Rinse in 70% alcohol; rinse in 95% alcohol; rinse in absolute alcohol 5 to 15 seconds.

5. Dip the slide into a staining jar containing 0.5% fast green in absolute alcohol. (The fast green may be used in 95% alcohol

[1]Haynes, Rachel. Fast green, a substitute for light green SF yellowish. Stain Techn., 3, 40. 1928.

[2]A National Aniline safranin (Cert. No. NS-7) and a Coleman and Bell fast green FCF (uncertified) were used by the writer.

and the schedule readjusted.) Move the slide gently to and fro 6 to 10 times, or until it is properly differentiated.

6. Rinse in absolute alcohol.

7. Dip slide in clove oil, and move it to and fro 6 to 10 times in order to get rid of the alcohol.

8. Rinse in xylol to remove the clove oil. Check slide under microscope to be sure the stain is right.

9. Clear in xylol.

10. Put on cover glass.

—JOHN ADAM MOORE, Henry Shaw School of Botany, Washington University, St. Louis, Mo.

A NEW PROCEDURE FOR THE FEULGEN REACTION; A PRELIMINARY NOTE.—The Feulgen stain (see Feulgen and Rossenbeck, Zts. Physiol. Chem., **135**, 203–48, 1924) has an established value in cytology, but is not in common use, partly because much of the basic fuchsin on the market is unsatisfactory, and partly because of difficulties in the technic. Attention is here called to certain changes in the technic which will be found of assistance.

1) Use of $K_2S_2O_5$ in dye solution and in acid rinse solution.

2) Use of distilled water thruout.

3) Increase in timing of steps to following:
   4–5 min. hydrolyzation
   2 hr. staining for animal tissue; 3–5 hr. for plant tissue.
   10 min. each in three successive baths of acid potassium bisulfite.

4) Use of fast green FCF or orange G instead of light green as counterstain.

By paying attention to these points and by taking care to use a batch of basic fuchsin showing decolorization in the Feulgen formula to a light straw color or faint pink, one can avoid many of the pitfalls of the technic. A later paper will discuss the procedure in more detail and give definite instructions as to how to obtain a suitable basic fuchsin.—J. A. DE TOMASI, Geneva, N. Y.

PRESENT MEMBERS OF THE COMMISSION ON STANDARDIZATION OF BIOLOGICAL STAINS

Altho it is only two years since the list of members of the Stain Commission was last published (April, 1934), there have been so many recent changes in personnel that publication of an up-to-date list seems desirable again. The names below constitute the membership of the Commission, as of March 1, 1936.

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R. B. H. Gradwohl, 3514 Lucas Ave., St. Louis, Mo.
F. W. Hachtel, 122 W. Lafayette Ave., Baltimore, Md.
W. A. Hagan, Cornell University, Ithaca, N. Y.
Frank E. Hale, Mt. Prospect Lab., 421 Flatbush Ave., Brooklyn, N. Y.
Robert T. Hance, Dept. of Zoology, Univ. of Pittsburgh, Pittsburgh, Pa.

H. T. Herrick, Bureau of Chemistry & Soils, U. S. Dept. of Agric., Washington, D. C.
D. C. Hetherington, Duke Hospital, Durham, N. C.
E. G. Hood, Dairy & Cold Storage Branch, Dept. of Agric., Ottawa, Canada.
Davenport Hooker, Univ. of Pittsburgh, Pittsburgh, Pa.
Alfread E. A. Hudson, City Memorial Hospital, Hickory, N. C.
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S. H. Hutner, Dept. of Physics, Mass. Inst. Tech., Cambridge, Mass.
D. A. Irwin, Banting Institute, Toronto, Canada.
O. A. Johannson, 203 Parkway, Ithaca, N. Y.
B. F. Kingsbury, 2 South Ave., Ithaca, N. Y.
Max Levine, Iowa State College, Ames, Iowa.
Leon S. Lippincott, P. O. Box 303, Vicksburg, Miss.
H. Macy, University Farm, St. Paul, Minn.
W. L. Mallmann, Dept. of Bact., Mich. State College, East Lansing, Mich.
M. S. Marshall, Hooper Foundation, 2nd & Parnassus Aves., San Francisco, Calif.
Barbara McClintock, Botany Dept., Cornell University, Ithaca, N.Y.
C. E. McClung, Univ. of Pennsylvania, Philadelphia, Pa.
Roy L. Mobley, P. O. Box 1021, Baton Rouge, La.
John T. Myers, 5214 Fort Street, Omaha, Nebr.
Frederick Proescher, Box 864, San Jose, Calif.
L. F. Randolph, Plant Science Bldg., Ithaca, N. Y.
H. D. Reed, McGraw Hall, Ithaca, N. Y.
L. F. Randolph, 514 S. Comstock Ave., Whittier, Calif.
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A. E. Stearn, 1509 Rosemary Lane, Columbia, Missouri.
W. N. Steil, 1217 W. Wisconsin Ave., Milwaukee, Wis.
K. A. Stiles, Coe College, Cedar Rapids, Iowa
F. W. Tanner, 362 Chemistry Bldg., Urbana, Ill.
Wm. Randolph Taylor, Dept. of Botany, Univ. of Michigan, Ann Arbor, Mich.
Stanley Thomas, Lehigh University, Bethlehem, Pa.
J. M. Thuringer, 801 N. E. 13th St., Oklahoma City, Okla.
Alexander V. Tolstoouhov, 305 East 63rd Street, New York, N Y.
Seth T. Walton, City Health Dept., Charlotte, N. C.
Henry B. Ward, 1201 West Nevada Ave., Urbana, Ill.
H. C. Waterman, 2221 Randolph St. (Woodridge), N. E., Washington, D. C.
Thomas W. Whitaker, Agnes Scott College, Decatur, Ga.
B. G. R. Williams, Paris, Ill.
Conway Zirkel, Dept. of Botany, Univ. of Pennsylvania, Philadelphia, Pa.

LABORATORY HINTS
FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

The abstracts given here are intended primarily for laboratory use; consequently the technic in each instance is given in as much detail as possible.

J. A. de Tomasi — *Abstract Editor*

BOOK REVIEWS

PICKWORTH, F. A. **Chronic Nasal Sinusitis and Its Relation to Mental Disorder.** 7 x 10 in., 156 pp. Cloth. 83 illustrations. Index. Bibliographies. *H. K. Lewis & Co., London.* 1935.

This book is a comprehensive study of the subject under consideration. The text is supplied with a rich selection of photomicrographs and colored plates. Of special interest here is the "Appendix" because of some of the histological methods included, to wit:

Gram-positive organisms in tissues: Cut 10 μ paraffin sections (or 15 μ trichloracetic decalcified bone). Run down to water. Stain 5–15 min. in 1% aq. eosin. Wash 5 min. in water. Rinse in alcohol. Stain 15–30 min. in freshly made and filtered anilin methyl violet: 2% aq. methyl violet, 9 cc. and 20% anilin in abs. alcohol, 1 cc. Wash with Gram's iodine and hold therein 2–5 min. Blot and clear in several changes of anilin-xylene. Wash in xylene and mount in neutral Canada balsam or resin.

Gram-negative organisms in tissues: Treat sections overnight in 10% H_2SO_4. Wash 30 min. in running water. Stain 10–15 min. in Leishman's solution diluted 1:3 with dist. water. Wash in dist. water. Differentiate until pink in 1:1,500 acetic acid. Wash 3 min. in running dist. water. Stain 30–60 min. with Giemsa diluted 1:15 with 1% aq. pyridine. Wash in dist. water. Differentiate carefully in 1:3,000 acetic acid. Transfer to a mixture of equal parts of 1:200 tannic acid and 1:1,500 acetic acid. Wash in water. Dehydrate in acetone, clear and mount. Do not confuse organisms with occasional pigment granules of nerve cells or particles of corpuscular stroma of blood-clot.

Decalcification of sections containing bone: Cut thin (5 mm.) and treat (5 days to several weeks) with 5% trichloracetic acid in saline. Formalin fixation does not interfere. Abs. alcohol may hasten the process.

Stain for vessels in thick sections: Cut fresh brain fixed within 6 hr. after death into slices 1 cm. thick. Fix 2 days in formol saline: NaCl, 20 g.; 40% formaldehyde, 100 cc; water, 1,000 cc. Wash 24 hr. in water. Soak in gum phenol: gum arabic, 500 g.; phenol crystals, 20 g.; water 2,000 cc. (Warm to dissolve, filter thru wool and Seitz filter.) Cut 250 μ frozen sections. Wash 2 hr. in running water. Transfer for 1 hr. at 37° C. to sodium nitroprusside benzidine: sodium nitroprusside (nitroferricyanide) 0.1 g.; 0.5% benzidine in 2% acetic acid, 25 cc.; dist. water, 75 cc. Agitate the sections continuously in a shaking machine. Filter and use immediately. Rinse in water. Shake 1 hr. (37° C.) in dil. H_2O_2: 20 vol. H_2O_2 in 400 cc. water. Wash in water, dehydrate, clear and mount. An additional cellular stain such as dilute Giemsa may be introduced before clearing.—*J. A. de Tomasi.*

THE MICROSCOPE AND OTHER APPARATUS

ANGERER, K. and HEYER, E. **Ein Lichtfilter zum leichteren Auffinden der nach Ziehl-Neelsen gefärbten Tuberkelbazillen.** *Munch. med. Woch.*, **82**, 348–9. 1935.

A light filter is now available, made by Reichert (Vienna) upon the authors' suggestions, which helps to detect acid-fast bacilli. It is a combination of a yellow and violet filter which can be inserted beneath the Abbe condenser like any usual

blue glass filter. It will not work with daylight illumination. Ziehl-Neelsen stained acid-fast organisms stand out in ruby-red against a beige colored background. Other objects are bright green. (*Abstr.* in Zentbl. Bakt., II Abt., **92**, 506. 1935.)—*J. A. de Tomasi.*

DE ZEEUW, J. and KUENEN, D. J. Note on a micro-colorimeter and its possible application. *Protoplasma*, **23**, 626–9. 1935.

A simple device is prepared by mounting a semi-circular silvered cover slip at 45° in place of the fixed diaphragm in a microscope ocular. A 1 cm. hole is bored thru the side of the eyepiece opposite the mirror. In this way a horizontal light beam coming thru colored filters can be seen simultaneously with the object on the microscope stage.—*R. Chambers.*

RAMSTHALER, P. Ueber ein Mikroskopzusatzsystem mit Aperturblende. *Zts. Wiss. Mikr.*, **51**, 184–7. 1934.

All systems of side illumination as well as dark field condensers are at a disadvantage when compared with schemes of direct lighting and opaque illuminators. It is not possible to modify the aperture of the objective by means of a variable diaphragm. The author has developed an optical addition which allows projection at a suitable point within the microscope of both the virtual image of the object and the image of the frontal lens diaphragm. Here an iris diaphragm is built in, acting as an aperture diaphragm, which must be centered in each microscope to the image of the front lens diaphragm of the objective. The system is on the market as "Schrägblickaufsatz mit Irisblende" by Reichert, Vienna.—*J. A. de Tomasi.*

SCHARRER, B. Ueber die Feststellung von Dickenunterschieden an lebenden mikroskopischen Objecten, dargelegt am Beispiel der Unterscheidung von Hühner- und Rekurrensspirochäten. *Arch. Protistenkunde*, **85**, 87. 1935.

Dark-field microscopy affords a highly sensitive means for distinguishing organisms that differ very slightly in thickness. With immersion condensers of high N. A., objects of the same thickness as *Spirochaeta recurrentis* appear double-lined. With condensers of lower N.A., i.e., dry condensers, the objects appear single-lined. Thicker objects appear double-lined even with dry condensers; thinner objects appear single-lined even with condensers and objectives of the highest N.A. Of a large number of spirochaetes examined none had the dark-field optical properties of *S. recurrentis*, as most of them were thinner.—*S. H. Hutner.*

UBER, F. M. Illuminator for critical microscopy utilizing automobile headlight lamps. *Science*, **82**, 624–5. 1935.

Critical illumination for microscopy with high power objectives requires a small light source of uniform and high intensity. This can be obtained by focussing the light from a 6–8 v. headlight lamp on a finely ground glass used as a secondary source. In cases where the beam is insufficient for low-power field illumination, a supplementary lens may be inserted below the substage condenser. The intensity and size of the light beam can be easily varied by lowering a glass screen operated synchronously with the diaphragm.—*J. A. de Tomasi.*

PHOTOMICROGRAPHY

IVES, R. L. A simple method of determining areas in microphotographs. *Science*, **82**, 445–6. 1935.

Mechanical integration methods for determining areas in photomicrographs are slow and laborious. This method is based upon the principle that the weight of the photographic paper carrying the image of an object varies directly with the area of the image. Proceed as follows: Cut out of the print the image of the entire field (F) and weigh it. From the image of the entire field cut out the image (S) whose area is desired and weigh it. The desired area A_S is given by the equation $A_S = \frac{W_S A_F}{W_F}$ where W_S and W_F are the two weights determined and A_F is a numerical value obtained by integration. If the cutting is done carefully with a sharp blade, errors can be kept within 1%.—*J. A. de Tomasi.*

ROBINSON, W. L. **A simple photomicrographic camera.** *J. Techn. Methods & Bull. Int. Assoc. Med. Mus.*, **13**, 67–70. 1934.

This camera was designed to meet the needs for a recording apparatus in a routine laboratory. It is small, compact and as inexpensive as compatible with good performance. It is intended to be fixed permanently to the worktable along with the microscope and light. It consists of a box of seasoned white basswood, rectangular at its base, to accommodate 4″ x 5″ plate holders. Toward the top the box expands posteriorly to allow for a viewing aperture thru which focusing is done. There is also installed a right angle prism and a mirror set at 45° to deflect the image onto the base board at the bottom of the camera box. Focusing is similar to that of a Graflex camera. The main limitation is due to the fixed length of the bellows extension—a feature introduced for the sake of rigidity and simplicity in design.—*J. A. de Tomasi.*

DYES AND MICROTECHNIC IN GENERAL

GAILLARD, P. J. and HECHT, E. **Ein Beitrag zur Vereinfachung gewebezüchterischer Kulturmedien.** *Protoplasma*, **23**, 1–13. 1934.

A dried petrol ether (ligroin) extract of pig's brain, after thoro extraction with acetone, furnishes a powder which, in very low concentrations, takes the place of embryonic juice for coagulating the plasma in tissue cultures. The authors maintain that this method is as good as the usual one.—*R. Chambers.*

LISON, L. **Sur la détermination du pH intracellulaire par les colorants vitaux indicateurs. "L'erreur métachromatique."** *Protoplasma*, **24**, 453–65. 1935–36.

The author lists a series of metachromatic dyes from which he selected neutral red, brilliant cresyl blue, cresyl violet and Nile blue as examples of basic dyes which are used frequently as pH indicators (ionic virage) and which also shift color in the presence of chromotropic substances (metachromatic virage). These dyes were prepared in a concentration of M/10,000 in Sörensen's buffer solutions diluted to 1 part in 5 parts water. The pH color reactions of brilliant cresyl blue, cresyl violet and Nile blue agree with those stated by Fauré-Fremiet but not with those by Spek. The author also prepared similar solutions of the dyes in McIlvaine's buffer solutions from pH 2.2 to 8.0 (disodium phosphate, citric acid) diluted 1:6, to which was added a given amount of calcium chondroitin sulfate (prepared according to the procedure of Sawjalow). Other chromotropic substances were also used, e. g., agar-agar, the jellied extract of *Chondrus crispus*, cellulose, glycogen, gum arabic and chitin. It is significant that all these substances had no effect on the pH colors of Clark and Lub's indicators, or the universal indicator, B.D.H. For brilliant cresyl blue, with calcium chondroitin sulfate in concentration of 1/20,000, the metachromatic color, in solutions of pH 2.0 and 3.0, correspond to the color of pure buffer solutions at pH 10.4 to 11.4. Cresyl violet and Nile blue give similar metachromatic errors. Neutral red in the presence of 1/20,000 calcium chondroitin sulfate gives, at pH 2.2 to 6.5, the colors characteristic for pH 7.0 to 7.4.—*Author's Abstract.*

MAYERSON, H. S. **Standardization of photochemical methods for the measurement of solar ultraviolet radiation.** *Amer. J. Hyg.*, **22**, 106–36. 1935.

The bleaching of acetone-methylene-blue solutions is not an ideal method for measuring solar radiation, but it is the simplest. The reaction depends on the decomposition of an aq. solution of acetone by ultraviolet radiation. It is partly an oxidative process and is accompanied at the same time by reduction processes which are measured by the addition to the solution of methylene blue which acts as a hydrogen receptor. The bleaching of the latter is proportionate to the time of exposure. The English standards as proposed by Webster, Hill and Eidinow (Lancet **206**, 745–7, 1924) were duplicated as follows: Standard solution No. 10 (matching the standard acetone-methylene-blue solution) was made by adding 1 cc. of dist. water to each 2.4 g. of C. P. copper nitrate, previously dried in an electric oven for 24 hr. at 60° C., and filtering. Standard solutions No. 9 to No. 5 were made by diluting solution No. 10 with 0.05, 0.15, 0.30, 0.65 and 1 cc. of dist. water, respectively, for each cc. of solution. For standard solutions No. 4 and No. 3, solution No. 10 was diluted per cc. with 2 and 5 cc., respectively, of a 0.01% solution of K_2CrO_4. Duplicate sets of standards were prepared and

sealed in glass tubes of 3 mm. internal bore, and when not in use kept in the dark. Standard methylene blue solutions (No. 10) have also been prepared using 9 cc. of a 0.1% solution of medicinal methylene blue. (Schultz No. 659, National Aniline and Chemical Co.,) or 15 cc. of a 0.1% solution of De Haen methylene blue 2B, diluted with 30 cc. of Merck's C. P. acetone and made up to 100 cc. with dist. water. The solutions completely absorb all radiations shorter than 305 mμ and absorb about 90% of the radiation shorter than 320 mμ.

For the test, standard solution No. 10 is exposed vertically to the sun in a standard quartz tube of 55 mm. length and 3 mm. internal diameter, and the amount of fading measured by comparison with a set of standard tubes made up as previously described.

The method yields only very approximate values even under the most carefully controlled conditions. A glass control tube should always be exposed simultaneously with the quartz tube to measure the amount of bleaching in the visible spectrum. This amount should be deducted from the total bleaching under quartz. If the bleaching is too rapid, the tubes should be changed every 4 hr. Temperature corrections should be applied.—*G. H. Chapman.*

POPE, M. N. **A bath for orienting objects in paraffin.** *Science,* **82,** 356. 1935.

An embedding bath is described which will keep the paraffin melted until orientation is complete and will allow it to harden rapidly without change of position. It consists essentially of an open copper box with inlet and outlet pipes at opposite corners controlled by suitable stopcocks. The box is mounted horizontally on a tripod stand, and its inlet connects with a funnel held vertical above it. A copper sheet bent to reach the bottom of the box makes a platform on which paper trays with melted paraffin and objects can be kept warm. About 250 cc. of hot water in the copper box can be easily replaced with as much ice water, and hardening will occur in about 1 min.—*J. A. de Tomasi.*

RICHARDSON, G. D. **An improved rapid celloidin method of embedding.** *J. Techn. Methods & Bull. Int. Assoc. Med. Mus.,* **13,** 81. 1934.

This method is intended to eliminate shrinkage of the sections. Cut tissue no more than 2 mm. thick. Carry out all the following steps in a water bath at 55° C. Fix 2 hr. in 10% formalin. Dehydrate 2 hr. in acetone. Clear ½ to 2 hr. in clove oil. Infiltrate 3 hr. in celloidin. Block and harden in chloroform ½ to 2 hr. The celloidin is prepared as follows: add 1100 cc. abs. alcohol to 8 oz. air-dried celloidin and allow to stand over night. Add 1100 cc. ether and hold until dissolved.—*J. A. de Tomasi.*

SCANLAN, J. T. **The magenta series. I. The preparation and spectrophotometric study of the lower basic members.** *J. Amer. Chem. Soc.,* **57,** No. 5, 887. 1935.

An attempt to correct some of the variations of basic magenta (fuchsin) was made. It was found that this stain contains different compounds such as intermediates that are undesirable. An improved method of preparation for two of the lower homologs was devised. Spectrophotometric methods were used to identify and evaluate the commercial samples studied.—*Roy L. Mobley.*

STEWART, M. J. **A concentration method for the demonstration of asbestos bodies in the sputum.** *J. Techn. Methods & Bull. Int. Assoc. Med. Mus.,* **13,** 70–1. 1934.

About 15 g. of sputum are dissolved in an equal amount of antiformin by holding at 37° C. and shaking. From 60–90 cc. of water are added and the mixture allowed to stand a few hours. All but 12–15 cc. of the supernatant liquid is then decanted and the residue is moderately centrifuged for 10 min. After decanting and draining, the deposit is mixed thoroly but gently with a fine pipet and transferred to albuminized slides. Usually the whole sediment from a 15 g. sample may be spread over an area of 1 sq. inch. Dry on hot plate, fix over a flame, wash gently in water. Dry and mount in Canada balsam. Before mounting, the film may be treated with $K_4Fe(CN)_6$ and HCl to demonstrate the Prussian blue reaction given by the asbestos bodies; or, when black, they may be bleached with 5% $(NH_4)_2S$.—*J. A. de Tomasi.*

SYMEONIDIS, A. **New uses of the cold-knife frozen-section method for fixed tissue.** *Zentbl. allg. Path.*, **63,** 245–6. 1935.

With the knife cooled below freezing, better frozen sections can be obtained from fixed as well as unfixed tissue. Fixed tissue sections tend to come off the slide, hence, should be applied to an albuminized slide, smoothed out, and blotted carefully with several thicknesses of dry filter paper or filter paper moistened with 50% alcohol. Transferring the preparation to abs. alcohol for 30–60 sec. coagulates the albumin and tends to prevent separation of the section. Subsequent processing can be the same as for paraffin sections. Special stains (e. g., fat) or the oxidase reaction can be applied after fixing sections for 1 min. in 40% formalin.—*H. A. Davenport.*

THORNTON, H. R. and SANDIN, R. B. **Standardization of the methylene blue reduction test by the use of methylene blue thiocyanate.** *Amer. J. Pub. Health,* **25,** 1114–7. 1935.

Following the Standard Methods of Milk Analysis, 3 samples of methylene blue were tested in milk: standard American reductase test tablets of methylene blue (chloride), Danish tablets (also the chloride), and methylene blue thiocyanate prepared by the authors. Considerable variation in color depth were found even between different tablets in the same lot. Methylene blue thiocyanate was much more constant. The inadequacy of present empirical dye standards is due largely to the difficulty of purification and determination of purity of methylene blue. The following recommendations are made: (1) That methylene blue thiocyanate be substituted for methylene blue chloride in the standard tablets used in the methylene blue reduction test because of the reproducibility of the former dye; (2) That one part of dye to 300,000 parts of milk be adopted as the standard concentration in this test.—*M. W. Jennison.*

ANIMAL MICROTECHNIC

BERBLINGER, W. and BURGDORF. **Neue Färbemethode zur Darstellung der Gewebsbestandteile der Hypophyse des Menschen.** *Endokrinologie,* **15,** 381–8. 1935.

The new technic described for the demonstration of the various components of the hypophysis consists of the following steps: Fixation in 4–10% aq. or alc. formalin; hardening and dehydrating in graded alcohols; chloroform; paraffin. The sections, after removal of paraffin, are stained for 2–24 hr. in an alc. solution of cresofuchsin (0.5–1%), depending upon the strength of the stain, washed briefly in dist. water; then in alum-carmine for 3 hr.; rinse in dist. water, differentiate and stain the acidophil cells for 5 min. in the following: 100 ml. of 1% phosphomolybdic acid solution containing 2 g. orange G.; rinse in dist. water; place in 5% phosphomolybdic acid for 2 min.; dry with blotting paper; stain for 10–20 min. in (dist. water 100 ml., 0.5 g. water soluble anilin blue, bring to boil, filter cold and dilute filtrate with 2 parts dist. water); rinse in dist. water; differentiate in 75% alcohol until stain no longer issues from section; dehydrate; xylol, balsam. Chief cells, blue to gray; pregnancy cells, blue with small vivid yellow granules; basophiles with coarse reddish-blue granules; epithelium of the pars intermedia and pars tuberalis variable; collagen fiber, intensive blue, glia fibers, blue-gray; axons occasionally nearly black.—*J. M. Thuringer.*

COVELL, W. P. and O'LEARY, J. L. **Vital staining methods applied to nerve degeneration.** *J. Techn. Methods & Bull. Int. Assoc. Med. Mus.*, **13,** 92–3. 1934.

Neutral red was found to be specific for altered myelin. It can be used in various ways: intravenously, subcutaneously, by perfusion, and immersion. The intensity of the reaction depends upon the amount and concentration of the dye. A teased piece of a peripheral stump in 12 min. gives optimum staining in a 1: 10,000 dilution of the dye. The extent and degree of degeneration can thus be quickly ascertained.—*J. A. de Tomasi.*

DUPRÈ, G. C. **Un nuovo metodo di colorazione tipo Mallory.** *Monit. Zool. Ital.*, **46,** 77–83. 1935.

Fix in Zenker's with formalin or acetic, or preferably in Ruffini III (Muller's fluid, 50 cc.; 1% chromic acid, 50 cc.; glacial acetic acid, 2 cc; corrosive sub-

limate, 1 g.). After Ruffini III remove bichromate with Kaiserling II, adding several drops of 25% KI. Embed in paraffin or celloidin-paraffin. Cut sections 5 to 8 μ thick. Stain with Ziehl's basic fuchsin prepared according to Gallego, or when the fixing fluid contains acetic acid, use the following formula: Basic fuchsin, 1.5 g.; water, 160 cc.; 95% alcohol, 40 cc.; allow to stand 48 hr. at 37° C. before using. To 15 cc. of anilin water add 10 drops of the dye and 5–7 drops of acetic acid. Stain from 1–10 min. and pass into the following solution: water, 50 cc.; formalin, 20 cc.; glacial acetic acid, 25 cc. for 5–10 min. Wash briefly in water and pass into 1% phosphomolybdic acid for 10 min. Wash thoroly in water for about 2 min. Counterstain with any one of the following solutions (A preferred): (A) dist. water, 100 cc.; toluidine blue, 0.25 g.; orange G, 4 g.; oxalic acid, 4 g. (B) dist. water, 100 cc.; methylene blue, 0.30 g.; orange G, 4 g.; oxalic acid, 4 g. (C) dist. water, 100 cc.; malachite green, 0.20 g.; orange G, 4 g.; oxalic acid, 4 g. (D) dist. water, 100 cc.; methyl green, 0.30 g.; orange G, 4 g.; oxalic acid, 4 g. In making up the stains, heat, cool and filter. Stain 1 to 2 min. or more, blot dry and pass directly into 95% alcohol to differentiate. If necessary give a preliminary treatment with a little anilin oil in 80% alcohol. There is good differentiation of epithelium, connective tissue (including reticular tissue), cartilage, bone, striations and sarcoplasm of skeletal muscle and central and peripheral nervous tissue.—*A. B. Dawson.*

ELLIOTT, K. A. C. and BAKER, Z. **The effects of oxidation-reduction potential indicator dyes on the metabolism of tumor and normal tissues.** *Biochem. J.*, **29**, 2396–2404. 1935.

This article is a study on the metabolism of thin slices of various tissues in the presence or absence of glucose as well as a number of dyes functioning as reversible oxidation-reduction systems. Dyes used were: 4 from the indophenol group (Eastman Kodak Co.); indigo carmine and two other sulfonated dyes (La Motte); Bindschedler's green and *m*-toluylene-diamine-indophenol (British Drug Houses); thionin (Coleman & Bell Co.); "prune" (Ciba and Co.); $ZnCl_2$—free methylene blue (Merck, Darmstadt); Nile blue A and cresyl violet (National Aniline & Chemical Co.). The tissues used were: kidney, brain, retina, testis, liver and tumor from rat and rabbit. Results: The majority of the dyes in a $10^{-3}M$ concn. accelerate the respiration of tumor tissue in presence of glucose. These dyes in higher concentrations inhibit respiration of the other tissues. Thionin, "prune," methylene blue and cresyl violet in $10^{-3}M$ concentration accelerate respiration of tumor slices in glucose medium, and increase aerobic glycolysis. In $10^{-5}M$ concentration, the effects of dyes are less marked.—*J. A. de Tomasi.*

HAYMAKER, W. and SÁNCHEZ-PÉREZ, J. M. **Rio-Hortega's double silver impregnation technique adapted to the staining of tissue cultures.** *Science*, **82**, 355–6. 1935.

A technic for silver staining of tissue cultures *in situ* in the plasma clot which has proven to be quick, simple and dependable has been developed as follows: After removing all paraffin and vaseline from cover slip, fix 24 hr. in a 1:1 mixture of 10% neutral formalin and normal physiol. saline. Wash for 5 min. in Petri dishes containing 30 cc. doubly dist. water, plus 6 drops NH_4OH, for thin clots on cover slips; up to 15 min. in Carrel flasks for thicker clots. Wash in dist. water. Transfer to the following: 2% $AgNO_3$, 30 cc.; 95% alcohol, 50 drops; pyridine, 25 drops; NH_4OH, 5 drops. This is heated slowly to 40° C. until a yellow color develops (about 12 min.). Wash in dist. water. Transfer to the following: Ag_2CO_3 solution, 30 cc.; 95% alcohol, 25 drops; pyridine, 15 drops. Heat slowly to 40° C. until the fragments take on a brown color (about 9 min.). Prepare the Ag_2CO_3 solution as follows: Add 20 cc. of 5% Na_2CO_3 to 5 cc. of 10% $AgNO_3$; dissolve the white precipitate by adding just enough NH_4OH, drop by drop, and make up to 75 cc. with dist. water. Wash in dist. water. Reduce in 1% formalin. Wash in dist. water. Tone in 1:500 $AuCl_3$. Heat slowly to 40° C. until a violet color develops (about 10 min.). Fix a few minutes in 1% "sodium hyposulfite". Run up thru the alcohols, xylol, balsam.—*J. A. de Tomasi.*

HINGST, H. E. **A note on making permanent preparations of anopheline midguts.** *Amer. J. Hyg.*, **22**, 278–82. 1935.

Fix the specimen for 5 min. in the following solution: abs. alcohol, 90 parts; formalin, 7 parts; acetic acid, 3 parts. This is best done by drawing the fixing

fluid under the cover slip on one side while drawing off the dissecting medium with a strip of filter paper on the opposite side. The gut is not thoroly permeated by the fixative until it becomes milky white in color. This should take only a few seconds. Cover the entire slide with the fixative for another 5 min. Carefully lift (not slide) the cover slip with the aid of dissecting needles. The gut usually adheres to either the slide or the cover slip, otherwise subsequent handling will be extremely difficult. Flush the specimen with 70% alcohol and then cover it with the alcohol for 5 min. Replace with 50% alcohol for 5 min. Stain with Weigert's iron hematoxylin for 30 sec. (Solution No. 1: hematoxylin crystals, 1 g.; 95% alcohol, 100 cc. Solution No. 2: liq. ferri sesquichloride, 4 cc.; dist. water, 95 cc. Just prior to staining take 0.5 cc. of each solution and add to 25 cc. of 50% alcohol.) Flush with 50% alcohol. Cover with 70% alcohol for 5 min. followed by 95% alcohol for another 5 min. Counterstain with a weak solution of Bordeaux red in abs. alcohol (weak enough so that the specimen will not overstain in 30 sec.). Flush with abs. alcohol. Cover with Euparal and a cover slip.—*G. H. Chapman.*

KAY, W. W. and WHITEHEAD, R. **The staining of fat with Sudan IV.** *J. Path. & Bact.*, **41**, 303–4. 1935.

Sudan IV (toluene-azo-toluene-azo-beta-naphthol), as prepared by the British Drug House Ltd., gives a darker stain for fats than Sudan III. The author prepares the following stock solution: 22 g. Sudan IV to one liter abs. alcohol, boiled gently until dissolved. For use, add 9 parts (by volume) of this stock solution to 7 of 45% alcohol, and filter after standing 1 hr. Use while quite fresh. The technic as applied to formol fixed frozen sections is as follows: 50% alcohol, 5 min.; Sudan IV (prepared as above) 30 min. at 37° C., turning sections once; 50% alcohol, a few seconds; dist. water, a few minutes; filtered hemalum (time unspecified); tap water, several minutes; mount in glycerin jelly.—*V. Warbritton.*

KERNOHAN, J. W. **Adaptation of formalin-fixed tissue to Mallory's phosphotungstic acid hematoxylin stain.** *J. Techn. Methods & Bull. Int. Assoc. Med.*, **13**, 82–4. 1934.

Formalin fixation is not conducive to good results with differential staining. The following technic overcomes this difficulty: Wash the formalin fixed tissue in running water or "ammonia water" for a short time. Fix 4 days in Weigert's primary mordant ($K_2Cr_2O_7$, 5 g.; CrF_3, 2 g.; water, 100 cc.). Fix 2 days in Weigert's secondary mordant: (copper acetate, 5 g.; CrF_3, 2.5 g.; 36% acetic acid, 5 cc.; formol, 10 cc.; water, 100 cc.). Embed in paraffin and stain as usual with any one of Mallory's differential methods.—*J. A. de Tomasi.*

MAHDIHASSAN, S. **Further studies on the symbiotes of scale insects.** *Arch. Protistenkunde*, **85**, 61. 1935.

For permanent preparations of the symbiotic microörganisms of scale insects proceed as follows: Brush powder off insect. Place insect on a slide in a drop of a mixture composed of equal parts of glycerin, lactic acid and water. Rupture body with a fine lancet. After exudation of sufficient symbiont-containing body fluid, remove insect skin and place a cover slip on drop. Blot edges and apply a layer of quick-drying oil varnish. After a few days, when varnish is dry and hard, repaint the edge with Canada balsam over the oil varnish. Such preparations have remained unchanged for 9 years, even when stained with cotton blue.

To show the entire microflora of a young insect body, fix the larvae in modified Carnoy's fluid (abs. alcohol, 60 cc.; chloroform, 30 cc.; glacial acetic, 5 cc.; formol, 10 cc.) for 10 min. Change gradually to water. Treat with 5% KOH in the cold until the skins are quite transparent; longer treatment injures later staining. Wash cleared skins in water, treat with dilute acetic and finally transfer to alcohol. Stain with acid fuchsin or alc. eosin. Later treat with xylol containing a trace of picric acid until the skins under the microscope are just becoming yellowish. Wash in xylol and mount in Canada balsam. The microflora stands out bright red against the pale orange of the skin. A splendid photomicrograph of an insect *in toto* shows the microflora very clearly. (Staining formulae are not given. Strength of alcohol is not stated.)—*S. H. Hutner.*

McCORD, C. P., HOLDEN, F. R. and JOHNSTON, J. **Basophilic aggregation test in the lead poisoning epidemic of 1934–1935.** *Amer. J. Pub. Health*, **25**, 1089–98. 1935.

The presence of excess numbers of basophilic erythrocytes in human blood is indicative of lead poisoning. Search for these forms is best made in laked cells, after staining. A modified Manson's methylene blue yielded most consistent results, the formula being: borax, 1 g., methylene blue, 2 g., boiling dist. water, 100 cc. The borax is dissolved in the water and to this is added the methylene blue. After filtering this stain is ready for use and provides a stable, satisfactory, and uniform stain for at least 2 weeks.

Thin, even blood smears are made on slides and allowed to dry. The proper drying of these smears is important; ordinarily the optimum time lies between 1 and 3 hr. After drying, ½ of the slide is overlaid by a strip of filter paper, and cautiously there is applied with a pipet or dropper the minimum amount of methyl alcohol required to moisten the filter paper until it clings to the slide. This is allowed to dry until the filter paper becomes loose.

The slide is then immersed in a Coplin jar, containing the modified Manson stain, for approximately 10 min. It is impossible to overstain. After staining, the slides are washed in 3 or 4 changes of dist. water. Air drying is recommended.

For counting, an oil immersion objective and a 10× ocular with a Whipple grid are used. The basophilic aggregations in 20 fields of the unfixed portion of the slide are expressed as a percentage of the number of red blood cells counted in the fixed portion (average of 5 fields × 4).

Basophilic aggregations over 1–1.5%, and especially over 2%, in persons exposed to lead, suggest lead absorption.—*M. W. Jennison.*

MENKE, J. F. **The hemolytic action of photofluorescein.** *Biol. Bull.*, **68**, 360–2. 1935.

A photocompound of fluorescein was made by continuous irradiation of a dilute solution of sodium fluorescein: dye, 50 g.; dist. water, 2 liters. By means of a 4½-in. reading glass, the sun's rays were focused for 300 hr. on an Erlenmeyer flask containing the solution.

By the addition of a small amount of HCl (quantity not stated) the photocompound was precipitated and, after washing with dist. water, the dye was reconverted into its sodium salt by adding enough NaOH to redissolve it. The solution was then evaporated to dryness and 1 mg. of the residue dissolved in 0.1 cc. of Locke's solution. The pH was found to be 7.85.

The red blood cells of a healthy rat were washed with Locke's solution. Alkaline buffers and a water bath of 38° C. were used in each experiment.

In 9 series of experiments photofluorescein produced hemolysis in the dark just as fluorescein does in the light. Unirradiated fluorescein had no hemolytic action in the dark. Hemolysis occurred only in the buffered controls of pH 10.0, and this was slight. It required 2 hr. in the dark for a 1:2,000 dilution of the photocompound to hemolyze 1 cc. of 1% red blood cells.

Long periods of irradiation are necessary to form appreciable quantities of photofluorescein, but shorter periods will produce hemolysis when the blood cells are in contact with the dye or when the fluorescein is previously irradiated.—*W. R. Hunt.*

MENNER, E. **Ganglienzellen-Präparate für Kurszwecke.** *Zool. Anzeiger*, **110**, 200–2. 1935.

For classroom preparations of multipolar ganglion cells, the author proceeds as follows: Use fresh spinal cord of pig. With scissors remove as much of the white substance as possible. Pieces of the gray substance 1–2 cm. long are placed into 50 cc. of macerating fluid (chromic acid solution 0.05%, 5 cc.; dist. water, 45 cc.) from 2–8 days depending upon temp. The following staining mixture is said to keep well: azoacid blue B (Hoechst),2 g.; tartar emetic, 1 g.; oxalic acid, 4 g.; dist. water, 200 cc. brought to a boil, allowed to cool and filtered after 24 hr. The tissue is transferred to a dilute stain consisting of 4 parts of the above in 96 parts of dist. water for 24 hr., is rinsed rapidly in dist. water and placed for 24 hr. in 1:3 glycerin water. It is then transferred to 1:1 glycerin water for 24 hr., and placed in pure glycerin for 2 weeks where the staining reaches its optimum. Small pieces are then transferred to a drop of glycerin on a slide and gently flattened

between the slide and cover glass. The cells appear brilliant blue with dark blue nuclei. Nissl granules show clearly, axons and dendrites are pale blue. The method is said to yield permanent results.—*J. M. Thuringer.*

MERLAND, A. **Méthode simple et rapide pour l'imprégnation des astrocytes fibrillaires.** *Bull. d'Histol. Appl.*, **12**, 290–1. 1935.

This method is useful in demonstrating the astrocytes of the neuroglia fibrils, with their extensions and "trompes vasculaires". The steps of the schedule are: Fix small pieces 6 days to 1 year in the following solution: NH_4Br, 10 g.; $Co(NO_3)_2$, 2 g.; neutral formol, 15 g.; dist. water, 100 g. Wash in dist. water. Cut frozen sections and wash several times in the following: Co $(NO_3)_2$, 2 g.; neutral formol, 10 g.; dist. water, 100 g. To make sure that all NH_4Br is eliminated, test with $AgNO_3$. Mordant sections in the same liquid 30–45 min. at 56° C. Wash rapidly in dist. water. Impregnate in 10% $AgNO_3$ at 56° C. until ochre yellow (about 30–60 min.). Reduce in the following: hydroquinone, 3 g.; formol, 10 g.; dist. water, 100 g.; anhyd. Na_2SO_3, 0.20 g. A black precipitate in this bath indicates insufficient washing of sections; these should turn almost black. If they are too light, mordanting was insufficient. Toning may follow in 0.20% $AuCl_3$, or sections may be fixed directly in 10% Na_2HSO_2. Wash, dehydrate, mount in balsam.—*J. A. de Tomasi.*

MOSER, F. **Wurstuntersuchungen nach Brekenfeld.** *Zts. Fleisch- Milchhyg.*, **45**, 304–10. 1935.

For the examination of sausage meat, not only bacteriological but also histological methods are necessary. The following histological technic was found satisfactory. Cut off from various parts of the specimen at least 3 cubes having faces 1 to 1.5 cm. Fix 24 hr. in 10% formalin, wash 1 hr. in water and cut frozen sections. Liver sausage, bologna and overdried material do not make satisfactory sections. Paraffin embedding is to be preferred. Stain with a 2% aq. methylene blue solution.—*J. A. de Tomasi.*

OPPENHEIMER, J. M. **Localized vital staining of teleostean embryos.** *Science*, **82**, 598. 1935.

The technic of Vogt (*Arch. Entwmech.*, **106**, 542–610, 1925) is modified by employing stain-impregnated cellophane, instead of agar. The cellophane is impregnated by treating with a 1 to 5% solution of the dye. It is not a bacterial medium, does not swell, is much more rigid and easier to handle than agar. Nile blue sulfate and neutral red are both satisfactory for staining the teleostean blastoderm, either in normal or double-strength Ringer's solution. Nile blue sulfate remains definitely localized for a longer time when used in combination with neutral red. In work on *Fundulus*, best results were secured by staining the blastoderms with cellophane treated in a mixture of equal parts of 1% solutions of the 2 dyes.—*J. A. de Tomasi.*

OSGOOD, E. E. and WILHELM, M. M. **Reticulocytes.** *J. Lab. & Clin. Med.*, **19**, 1129. 1934.

A simple technic was worked out for reticulocyte staining which gives uniformly good results and significantly higher counts than other methods tested. Mix in a small test tube equal parts (5 drops) of oxalated venous or capillary blood and 1% brilliant cresyl blue in 0.85% NaCl solution. Let stand 1 min., mix, and make thin smears, drying in air. These smears may be counted at any time within 24 hr., but if a permanent preparation is desired, they should be counterstained with Wright's stain by the usual technic. Count all red cells and all reticulocytes in as many adjacent fields as are necessary to give a total of 500 red cells if the count is over 5%, or 1000 cells if the count is less than 5%.—*E. Bachelis.*

PENFIELD, W. **A technique for demonstrating the perivascular nerves of the pia mater and central nervous system.** *Amer. J. Path.*, **11**, 1007–10. 1935.

Wash out blood by saline perfusion. Fix with a solution of 10.5% citric acid (powder) in 20% formalin. Inject fixative or leave in for 2–3 days. Dissect out blood vessels by the aid of a dissecting microscope. Wash in 2 changes of dist. water and put into 20% aq. $AgNO_3$ for 2 hr. Follow by 4 changes of 20%

formalin, of 100 cc. each. Pass to ammoniacal $AgNO_3$ (add 28% concentrated ammonia to 20% $AgNO_3$ drop by drop until precipitate redissolves, then add 3 drops excess). If tissue turns black add more ammonia to control rate of staining as determined under microscope. When staining is adequate put into 20% ammonia water 1 to 2 min. Wash in dilute acetic acid (concentration not given) to counteract alkalinity. Tone with gold, fix, wash, dehydrate, clear and mount. —*H. A. Davenport.*

PEREIRA, C. and VAZ, Z. **Ganzeinbettung von Nematoden; neue und einfache Einbettung in Balsam.** *Arch. Inst. Biol. Sao Paulo*, **5**, 77–86. 1934.

Various methods of preparing nematodes for microscopical examination are given. (1) For material treated with any fixative suitable for a carmine stain: hold 2–12 hrs. in Semichon's acetic carmine, differentiate 5–15 min. in 1% HCl in 70% alcohol, dehydrate 5–10 min. in abs. alcohol. (2) For material treated with acetic acid suitable for picric acid staining: hold 5–10 min. in 1:150 picric acid in 80% alcohol, dehydrate 5–10 min. in abs. alcohol. (3) For material treated with acetic acid and not to be stained: dehydrate 5–10 min. in abs. alcohol. (4) Embedding technic: Immerse the stained sections in 5% celloidin, 1 min., dehydrate 3 min. in 95% alcohol, clear 20 min. in beechwood tar creosote. Mount in syrupy Canada balsam. (*Cited from* Zentbl. Bakt., I Abt., Ref., **119**, 299. 1935.)—*J. A. de Tomasi.*

RIEMER, B. **Some practical suggestions in the preparation of bone for histologic study.** *J. Techn. Methods & Bull. Int. Assoc. Med. Mus.*, **13**, 72–6. 1934.

Fixation: For the best preservation of cellular structure a chrome fixative (such as Helly's fluid) is found to be most satisfactory. Formalin can be used for soft or young bone, or when decalcification with HNO_3 can be accomplished within 24 hr., thus allowing satisfactory nuclear staining.

Decalcification: Ideal decalcifier is 5% aq. HNO_3, used at 37° C. and shaken at least twice daily. The blocks should never be thicker than 2–3 mm. and 3–4 days treatment should suffice. Nine-tenths of the decalcification takes place in the first 24 hr.

Embedding: Young or spongy bones do well in paraffin, large pieces or cortical bone of adults are better in celloidin. The latter technic is carried out as follows: 46 hr. in thin (oil-like) celloidin; 48 hr. in thick (syrupy) celloidin. Transfer pieces to a large dry Petri dish and pour over a thick solution of the same celloidin. Air bubbles disappear later during hardening, which occurs in 48–72 hr., yielding a material of even consistency thruout. When this reaches a rubbery consistency, cut out in blocks, trim, and soak 24 hr. in 80% alcohol.

Spreading: Paraffin sections tend to loosen from the slide. After decerating, pass thru abs. alcohol and dip into a very thin celloidin solution. Wipe off excess liquid from back and edges of slide and transfer to 30% alcohol until ready for staining. Celloidin may be removed with alcohol-ether or by clearing 2 or more hr. in clove oil.

Staining: Celloidin sections are better stained loose by transferring with a spatula to a hematein solution. Mayer's hematein (hemalum) is most satisfactory. Ripen this for a week and stain for 6–12 hr. Overstaining, shown by uniform blue color, can be corrected with acid alcohol, following carefully the decolorization of the cement line structures. Wash 3 hr. in running tap water. Stain 10 min. in 1% aq. eosin, transfer to water, 80% alcohol, and 2 changes of 95% alcohol. Fish sections onto albuminated slides, press between lens paper, transfer for 3 hr. to clove oil. Pass into abs. alcohol for a few minutes, then xylol and mount.—*J. A. de Tomasi.*

RULON, O. **Differential reduction of Janus green during development of the chick.** *Protoplasma*, **24**, 346–64. 1935–36.

Chick embryos at various ages were removed from the egg and stained for about 8 minutes with Janus green in a 1/50,000 concentration in modified Locke's solution (0.9% NaCl, 0.042% KCl, 0.024% $CaCl_2$). They were then washed and sealed under a cover slip in the salt solution, care being taken to eliminate all air bubbles from the sealed chamber. The rate, intensity on regions of reduction of the dye were noted, the reduction changing the color from light blue to red. The yolk digesting cells in the area opaca were the most active in re-

duction. Different regions showed active reduction at different ages, the differentials in reduction corresponding closely to disintegration gradients of Hyman (1927) and Hinrichs (1927).—*Author's Abstract.*

STAFFORD, E. S. **An eosin-methylene-blue technique for rapid tissue diagnosis.** *Bull. Johns Hopkins Hosp.*, **55**, 229. 1934.

A new technic has been worked out and found to be satisfactory for rapid tissue diagnosis. Cut small thin blocks (not over 3 mm. thick). Heat to boiling in fixing solution (15% formalin, 75 cc., abs. alcohol, 25 cc.). Freeze and cut sections after washing blocks in water. Stain 1 sec. in eosin solution (eosin, 1 g., potassium bichromate, 1 g., water, 100 cc.). Wash in water. Stain deeply in methylene blue solution (Goodpasture) 10–20 sec. (methylene blue, 1 g., potassium carbonate, 1 g., water, 400 cc.; boil 30 min., add 3 cc. glacial acetic acid, shake until precipitate dissolves and concentrate to a volume of 200 cc.). Wash in water. Mount on slide. Blot (moisten blotting paper with drop of dehydrating fluid). Dehydrate with 15–20 drops of the following solution: abs. alcohol, 1 part, acetone, 5 parts. Dip slide into xylol. Mount in balsam.

The coloring and appearance of sections are similar to those of hematoxylin-eosin.—*E. Bachelis.*

SZEPSENWOL, J. **Un procédé d'imprégnation argentique applicable aux jaunes larves d'amphibiens.** *Compt. Rend. Soc. Biol.*, **116**, 1280–1. 1934.

Silver impregnation of young amphibian larvae has heretofore proved a difficult task even after lengthy fixation in 10–15% formalin. The author's method is: Fix material for at least 2–3 months, or better for a year, in 10–15% formalin or for 30 days in 15% formalin followed by 95% alcohol 10–15 days. Wash in running water for 24 hr. Pyridine, 10 days. Dist. water 24 hr. Place in 3% silver nitrate, 3–4 weeks in the dark at 28–30°. The larvae should appear dark brown with a reddish tinge. Wash rapidly in dist. water. Treat in 10% ammoniacal silver nitrate solution 6–8 hr. Wash rapidly in dist. water, and transfer to neutral 20% formalin. In successful impregnations, the larvae will retain their brown color, the formalin remaining clear. Otherwise return larvae for a few days into 3% silver nitrate, followed by the remainder of the treatment.—*J. M. Thuringer.*

TAMURA, O. **Ueber eigenartige Riesensternzellen von Urodelen mit nur supravital färbbaren Granulis.** *Folia. Anat. Jap.*, **13**, 573–6. 1935.

A new type of cell is described which is found in the peritoneum of certain amphibia (the Urodela). With needle and forceps the light membrane is separated from the belly wall as a pellicle which can be stretched out and secured to a cover glass. Immersed in water, the tissue shows star-like cell formations of a brown-yellowish color and beautifully ramified. Their bulk is made up of numerous fine bright granules. Any fixing fluid will cause the contour of cell and granules to disappear; the latter can only be revealed by supravital staining with certain dyes. Stain not longer than 30–60 sec. with methylene blue, toluidine blue, neutral red or Nile blue sulfate; rinse in water and examine in water immersion. The stained preparation can subsequently be fixed with formol or sublimate, rinsed, dried and mounted in balsam. Hematoxylin and eosin used on fixed material stain only the nucleus. As a differential characteristic it is found that polychrome methylene blue stains the granules of mast cells red violet and those of the giant star cells, blue.—*J. A. de Tomasi.*

WALLART, J. **Une modification de la méthode argentique de Bielschowsky, pour organes riches en lipoïdes.** *Bull. d'Histol. Appl.*, **12**, 254–6. 1935.

The observation that lipids and other fatty bodies in the ovary hinder the penetration of silver solutions, suggested the following technic: Fix at least 2 weeks in dilutions of neutral formol in dist. water 1:9, 1:4, or 1:2. Blocks of 3–5 mm. thickness are fixed again, using the following liquid: Equal parts of pyridine, ether, and abs. alcohol, 5 cc., 4% aq. NaOH 1 drop. Wash 12–24 hr. in running water, and for the same time in dist. water, changing every 2 hr. Impregnate 5 days at 36–37° C. in 1.5 to 3% $AgNO_3$. Rinse a few seconds in dist. water. Soak 24–36 hr. at room temp. in the following: 10% $AgNO_3$, 10 cc.; 40% NaOH, 5 drops; redissolve the precipitate by stirring with addition of NH_4OH, drop by drop, until only a few black specks are left; make up to 100 cc.

with dist. water. Wash 2 hr. in dist. water, changing every 30 min. Reduce 12–18 hr. in neutral formol diluted 1:9 with dist. water. Dehydrate rapidly with abs. alcohol, followed by toluene, then paraffin (this should take from 10–12 hr.). Cut 7–10 μ sections and treat with the gold solution. Best results were obtained on cat and sow ovaries.—*J. A. de Tomasi.*

WILDER, H. C. **An improved technique for silver impregnation of reticulum fibers.** *Amer. J. Path.*, **11**, 817–20. 1935.

Fix in 10% formalin, acetic-Zenker or formol-Zenker. Paraffin, celloidin, or frozen sections. Put material into 0.25% $KMnO_4$ or 10% phosphomolybdic acid for 1 min. Rinse in dist. water, transfer to a solution of concentrated HBr (Merck 34%), 1 part; dist. water, 3 parts, for 1 min. This can be omitted after treatment with phosphomolybdic acid. Wash in tap water and dip in 1% uranium nitrate (Na free) for 5 sec. or less. Wash in dist. water 10–20 sec. and impregnate in Foot's silver diammino hydroxide (Amer. J. Path., **5**, 223–38, 1929) for 1 min. Dip quickly in 95% alcohol and reduce 1 min. in the following solution: dist. water, 50 cc.; 40% neutral ($MgCO_3$) formalin, 0.5 cc.; 1% uranium nitrate, 1.5 cc. Wash, tone with gold, fix, wash, counterstain if desired, dehydrate, and mount.—*H. A. Davenport.*

PLANT MICROTECHNIC

DUFRENOY, J. **A method for embedding plant tissues without dehydration.** *Science*, **82**, 335–6. 1935.

Methylal, $CH_2(OCH_3)_2$, is a solvent for lipoids and dissolves in water. It is, therefore, a good intermediate between water and fats, and can be used to advantage in carrying plant tissues from killing fluid to paraffin. After fixation, wash in water and transfer thru the following series into paraffin, holding the material 1 hr. at each step: 1:1 methylal-water; methylal; methylal dehydrated and neutralized with anhyd. Na_2CO_3; 1:1 methylal-paraffin oil. Warm tissue on a water bath and transfer to paraffin of low melting point. Within an hour, transfer to grade of paraffin required for embedding. The finer details of structure are well preserved, the lignified tissues retain a soft waxy texture.—*J. A. de Tomasi.*

KOSTOFF, D. and KENDALL, J. **Irregular meiosis in Lycium Halimifolium Mill., produced by Gall Mites (Eriophyes).** *J. Genetics*, **21**, 113–5. 1929.

Description of phenomena observed in flower buds of the host, *Lycium*, attacked by mites before and during the occurrence of meiosis.

The material was studied both in aceto-carmine and in permanent preparations. For permanent preparations, the tissues were fixed in Bouin's solution as developed by Allen, and in a copper-Bouin solution (100 cc. Allen's modification of Bouin's solution plus 3 g. of copper oxide). The former was used to study the chromosomes and the latter to study the tissues. The preparations were stained by Heidenhain's iron-hematoxylin and orange G.—*R. W. Cumley.*

LAWRENCE, W. J. C. **The genetics and cytology of Dahlia species.** *J. Genetics*, **21**, 125–59. 1929.

The following species were examined: *D. variabilis, D. Merckii, D. Maxoni, D. coccinea, D. coronata, D. imperialis, Bidens atrosanguinea, Hidalgoa Wercklei.* Good pollen mother-cell divisions were not obtained from *D. Maxoni, imperialis,* or *coronata.* Apparently, the other species furnished good material.

The somatic counts were made from root tips. *D. variabilis* and *D. coronata* were fixed in Newton's modification of Flemming's fluid. The other species were fixed in a solution of 1% chromic acid, 60 cc.; 2% osmic acid, 20 cc.; 5% glacial acetic acid, 25 cc., which gave better results. Various fixatives were used for the pollen mother-cell material, the best results being obtained by Kihara's method (Carnoy 1 min., followed by Flemming).

By trimming off the outer bracts and quartering the capitulum the florets can be cut *en bloc* transversely or longitudinally. Quite good fixations were obtained by this method altho the fixing of individual florets gave more consistent results. Smear preparations were tried without success.

Embedded material of root tips was cut 10 μ thick, and anthers 14 μ. The sections were stained by Newton's gentian violet method.—*R. W. Cumley.*

NEWTON, W. C. F. and DARLINGTON, C. D. **Meiosis in polyploids. Part I. Triploid and pentaploid tulips.** *J. Genetics*, **21**, 1–15. 1929.

Material was fixed in Flemming's solution of the composition: 1% chromic acid, 60 cc.; 2% osmic acid, 20 cc.; 10% acetic acid, 25 cc.

Newton's method of gentian violet staining was used thruout. This consists of staining 3–10 min. in 1% supersaturated aq. solution of Grubler's gentian violet, rinsing in water rapidly, and then for 15–60 sec. in a solution of 1% iodine and 1% KI in 80% alcohol, differentiating rapidly in 2 washes of abs. alcohol and 2 washes of clove oil, and mounting from xylol. The method was of particular value in studying side views of metaphase where large multiple chromosome associations were formed.

In the tulips where smears are to be made of the anthers, it is possible to cut across the 4 loculi at one end of the anther and squeeze out the pollen mother-cells, which may then be smeared by drawing a second slide across the first. Fixations of the smears with osmic vapour did not give satisfactory results.

Tulips provide unusually good material for the study of the pollen mother-cell division. The nuclei are extraordinarily resistant to the damaging effects of fixation, and synizesis is not found in smears.—*R. W. Cumley.*

PERCIVAL, J. **Cytological studies of some hybrids of Aegilops Sp. + W heats, and of some hybrids between different species of Aegilops.** *J. Genetics*, **22**, 201–78. 1930.

Cytological investigations were made of the meiotic phase in the pollen mother-cells of all the hybrids. Carnoy's solution was employed for permanent preparations and gave exceedingly good results, even in the very fine spireme stage of early prophases. It was allowed to act for not less than 30 min., followed by careful washing with abs. alcohol to remove all traces of acetic acid.

Longitudinal and transverse sections were cut 12–14 μ thick, and stained with Heidenhain's iron-alum-hematoxylin.—*R. W. Cumley.*

STRUGGER, S. **Beiträge zur Gewebephysiologie der Wurzel. Zur Analyse und Methodik der Vitalfarbung pflanzlicher Zellen mit Neutralrot.** *Protoplasma*, **24**, 108–27. 1935.

Vital staining of *Trianea* root hairs with neutral red in aq. solution at a pH more acid than 6.4 is restricted to the cell wall. In more alkaline solutions the cell wall decolorizes and the stain accumulates in the vacuolar sap.—*R. Chambers.*

MICROÖRGANISMS

DELÉTANG, R. **Contribution a l'étude de la réaction de Gram.** *Les Presses Modernes, Paris.* 134 pp. 1933.

Part I is a critical study of the Gram stain technic and of the physical and chemical theories that attempt to explain it. Several methods are discussed at length, with their specific advantages and limitations. The role of microörganisms is also analyzed with respect to conditions of the culture and site of the reaction within the individual cells. Part II deals with the author's research on successive steps of the staining procedure and of the factors influencing it. These include a study of the significance of cellular lipids, nucleoproteids and lipoproteids and resistance of the cell to decoloration. The Gram reaction is attributed to the slight permeability of Gram-positive cells to alc. iodine and also to the adsorption of dye on the surface of the cytoplasmic micellae (especially those of nucleo-protein nature). It is brought about by a combination of the Gram complex with certain lipids, particularly lipoproteids, with unsaturated fatty acids. The site of the reaction is thought to be the cytoplasm rather than the membrane. The differentiation between bacteria suggests that distinct physico-chemical differences exist between Gram-positive and Gram-negative organisms. The Gram stain should be regarded, not only as a tinctorial reaction, but also as an aid to bacterial classification.—*J A. de Tomasi.*

GIEMSA. **Ueber eine bemerkenswerte Fehlerquelle bei der färberischen Darstellung der Schüffner-Tüpfelung.** *Münch. med. Wschr.*, 1975–6. 1935.

With tertian malaria it is found that the staining of the Schüffner granula (Schüffner stippling) is missing because the fixation has been performed with an

insufficiently purified brand of methyl alcohol. Two grades of methanol are produced by the Merck Co.; one labeled *purissimus* (acetone-free) has proven to be satisfactory, the other called *purus* has not. (*Cited from* Zentbl. Bakt., I Ref., **120**, 174. 1926.)—*J. A. de Tomasi.*

HAGELSTEIN, R. **The Mycetozoa.** *N. Y. Micro. Soc. Bull.*, **1**, *Feb.*, 1936.

The microscopical examination of these organisms, otherwise called Myxomycetes or slime molds, should first be made as an opaque object, with a few of the sporangia blown out by a small hand blower to free them from spores. Then these sporangia are picked off, placed on a slide with water under a cover glass and examined by transmitted light. If the air cannot thus be driven out, a mixture of water and alcohol will assist. Badly contracted spores may be swollen rapidly by the admission of a drop of 5% aq. KOH, altho this tends to alter the color of the spores of certain species. Permanent preparations may be mounted in glycerin, glycerin jelly, or Canada balsam.—*J. A. de Tomasi.*

HALBERT, V. A. **A study in the differentiation of Escherichia coli and Aerobacter aerogenes by the bacteriostatic action of organic dyes.** *J. Bact.*, **30**, 653–4. 1935.

To a basic medium of standard extract agar (pH 7.8) a series of water soluble dyes are added in the following concentrations: 1:500; 1:1,000; 1:3,333; 1:5,000; 1:10,000. One-half of each plate is poured with plain agar, the other half with the agar and dye. The plates are streaked with the cultures and read after 24 hr. incubation. Dahlia B, Janus green B, martius yellow, benzyl violet, and aldehyde green (source not given) favored the growth of *Escherichia coli*, but inhibited *Aerobacter aerogenes.*—*J. A. de Tomasi.*

KABELIK, J. **Eine Modifikation der Doldschen Färbung des B. diphtheriae.** *Prakt. lek.*, p. 207. 1935.

A modification of Dold's stain (Zentbl. Bakt., I Abt., Orig., **124**, 220, 1932; *abs.* Stain Techn., **8**, 40) for *B. diphtheriae* consists in the substitution of brilliant green for anilin green. It has proven of value in many cases of diphtheria and pseudodiphtheria. This stain, developed along the lines of the Gram stain, gives better tinting (green) of the diphtheria organism than of the pseudodiphtheria organisms (brown). If this method is supplemented by culture on Löffler's blood serum, the specific organisms can be easily detected and isolated in 24–48 hr. (*Cited from* Zentbl. Bakt., I Abt., Ref., **120**, 4. 1935.)—*J. A. de Tomasi.*

KOCJAN, L. **Einige Resultate mit der Färbung der Milzbrandbazillen nach der modifizierten Fothschen Methode.** *Jug. Veterinarski Glasnik, No.* 6, 1935.

In 5 cases out of 35 diagnoses of anthrax based upon cultural, staining, and biochemical characteristics, van Heelsbergen's modified Foth's stain proved far superior to all other methods. (Original article not seen, and no details of technic are given in abstract above cited.) (*Cited from* Zentbl. Bakt., I Abt., Ref., **119**, 385. 1935.)—*J. A. de Tomasi.*

KRAJIAN, A. A. **A rapid method of staining Spirochaeta pallida in single sections of tissue.** *Arch. Dermat. & Syphil.*, **32**, 764–7. 1935.

The following technic is suggested: Fix fresh blocks of tissue about 5 mm. thick in 10% formalin heated to 67° C. for 10 min. (not required for formaldehyde fixed tissues). Cut sections 5 to 10 μ on a freezing microtome. Place for 5 min. in a fresh 1% aq. solution of sodium cobalti-nitrite. Wash in two changes of dist. water. Place for 15 min. in the following solution previously heated and held at 67° C.: uranium nitrate, 1 g.; 85% formic acid C. P., 3 cc.; glycerin C. P., 5 cc.; acetone, 10 cc.; 95% alcohol, 10 cc. Wash in two changes of dist. water. Put in 0.75% aq. $AgNO_3$ 1 hr. at 67° C. Rinse rapidly in dist. water. Develop 3 min. in 2 cc. of the following solution: Hydroquinone, 0.31 g.; sodium sulfite, 0.06 g.; acetone, 2.5 cc.; neutral C. P. formalin, 2.5 cc.; pyridine, 2.5 cc.; satd. solution of mastic in 95% alcohol, 2.5 cc.; dist. water, 15.0 cc. Expose to light 15 min. before use. The solution keeps from 2 to 3 weeks in a dark cool place. Prior to use add 1 drop Mayer's fixative. While developing, work under a 60

watt lamp 4 ft. away. Wash a few seconds in dist. water. Return to the 0.75% $AgNO_3$ for 15 to 25 sec. Wash in 2 changes of dist. water. Draw onto slide and blot. Dehydrate in abs. alcohol 2 min. Clear in xylol 2 min. Mount in dammar resin. A control containing spirochaetes should be run simultaneously. The method can also be used for bacteria.—*G. H. Chapman.*

RIPPEL, H. **Fossil microörganisms in a permian salt deposit.** *Arch. Mikrob.*, **6**, 350–8. 1935.

The presence of bacteria in salt can be shown by the direct soil staining technic of Conn, Winogradsky and Rossi-Cholodny, using carbol-erythrosin (phenol 5%, erythrosin 2%, in water). The sample is washed, then dissolved in sterile redistilled water, and centrifuged. The sediment is dehydrated with 96% alcohol, again centrifuged, and then shaken with ether. The treatment with ether is necessary to remove minute oil droplets which take up the stain and may be confused with coccoid forms. The clear ether is decanted, a small amount of water added and heated to drive off the remaining ether. After usual drying and fixing in the flame, the sediment on the slide is stained for 24 hr. in carbol-erythrosin and washed with water.—*P. Esau.*

SEIFRIZ, W. and ZETZMANN, M. **A slime mold pigment as indicator of acidity.** *Protoplasma*, **23**, 175–9. 1935.

The yellow pigment of the slime mold, *Physarum polycephalum*, is an acid-aklaline indicator with a color range from deep red-orange to bright yellow-green. The pigment is dissolved out by shaking the organism in water. The colors are as follows: pH < 1.0, deep chrome; pH 1.6, deep orange; pH 3, golden yellow; pH 5.0, dark yellow; pH 6.2, light yellow; pH 7.0, sulfur; pH 8.0 green-yellow; pH > 8.0, yellow-green.—*R. Chambers.*

STONE, W. S. **A method of eliminating Blastocystis hominis from cultures of Entamoeba histolytica.** *J. Lab. & Clin. Med.*, **21**, 190–1. 1935.

Growth and reproduction of *E. histolytica* are occasionally impeded by the presence of the flagellate-like fungus *B. hominis*. The influence of various concentrations of neutral acriflavine was studied, leading to the following technic: Add to each of the mixed cultures 0.05 cc. of a dry sterile mixture of 2 parts powdered rice starch and 1 part powdered animal charcoal. Add enough 1:10,000 neutral acriflavine in Ringer's solution to the supernatant fluid of the culture on Boeck-Drbohlav medium to make a 1:50,000 solution. Incubate 24 hr. at 37° C. Transfer by repeating the same procedure. Prepare a sterile mixture of 1 part horse serum and 7 parts of the following modified Locke's solution: NaCl, 9.0 g.; $CaCl_2$, 0.24 g.; KCl, 0.42 g.; $NaHCO_3$, 0.2 g.; *N* lactic acid, 0.23 cc.: dist. water, 1,000 cc. Add 5 cc. of this mixture to egg slants and transfer the culture a few times. A final growth on Boeck-Drbohlav alone yields pure cultures of the amoebae.—*J. A. de Tomasi.*

ZEETTI, R. **Nouvelle méthode pour colorer facilement les spores des schizomycètes.** *Boll. Sez. Ital. Soc. Int. Micro.*, **7**, 310–1. 1935.

This new spore stain claims the advantage of eliminating the precarious step of decolorization usually resorted to for the purpose of differentiating between spores and vegetative forms. Proceed as follows: Fix the preparations by flaming. Flood with the following solution: Lugol's iodine, 100 cc.; eosin Y, 5 g.; phenol crystals, 5 g.; filter thru paper before storing. Heat for 5 min. (boil 2–3 times); boiling will cause no precipitation. Wash quickly in dist. water. Counterstain 1½–2 min. in sat. alc. methylene blue diluted 1:20 with dist water. Rinse, air dry and mount. Vegetative forms stain blue, spores a deep pink.—*J. A. de Tomasi.*

STAINS RECENTLY CERTIFIED

In the table below is given a list of the batches of stain approved since the last one listed in the January number of this journal.

STAINS CERTIFIED DECEMBER 1, 1935 TO FEBRUARY 29, 1936*

Name of dye	Certification No. of batch	Dye Content	Objects of tests made by Commission†	Date approved
Methylene blue	CA 15	86%	For histology, bacteriology, and as constituent of blood stains	Dec. 11, 1935
Cresyl violet	NW 5	97%	For use in histology	Dec. 13, 1935
Brilliant green	NBg 1	95%	For use in bacteriological media	Dec. 16, 1935
Crystal violet	DC 1	92%	For use in bacteriological media	Dec. 16, 1935
Gentian violet	CB 12	81%	For use in histology, bacteriology and cytology	Dec. 19, 1935
Methylene violet	NLv 3	——	As constituent of blood stains	Jan. 28, 1936
Orange G	NO 6	87%	As counterstain in histology	Feb. 6, 1936
Methylene blue	LA 6	84%	For histology, bacteriology, and as constituent of blood stains	Feb. 8, 1936
Carmine	NCa 5	——	For use in histology and cytology	Feb. 27, 1936

*The name of the company submitting any one of these dyes will be furnished on request.

†It is not to inferred that these are the only uses for which each of these samples may be employed. The Commission ordinarily tests each dye for such of its common uses as seem to give the most severe check as to its staining value. Certification does not in any instance, however, imply approval for medicinal use.

ERRATUM SLIP

The following error occurs in the paper by McWhorter and Weier in the July number (pp. 107–17):

The cuts for Fig. 4 (p. 112) and Fig. 6 (p. 115) are transposed, so that the legend under the latter refers to the former, and vice versa.